"PHARMACOGNOSTIC, PHYTOCHEMICAL AND INVITRO ANTILICE AND ANTIDANDRUFF ACTIVITIES OF *Dichrostachys cinerea* (L.) Wight & Arn"

Dissertation submitted to
The Tamilnadu Dr. M.G.R. Medical University, Chennai
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CERTIFICATE

This is to certify that the dissertation entitled **"Pharmacognostic, Phytochemical and Invitro Antilice, Antidandruff activities of *Dichrostachys cinerea* (L.) Wight &Arn"** was done by **Mrs. M. Vijayalakshmi** in Department of Pharmacognosy, Madurai Medical College, Madurai – 20, in partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmacognosy, under my guidance and supervision.

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Chapter - I
INTRODUCTION

Medicinal Plants

Medicinal plants have been an integral part of human civilization from time immemorial. There has been an increasing awareness in recent years in ethnobiological studies mainly on the traditional medicine and particularly on tribal medicine, because of their time tested efficacy without side effect.

WHO estimates that worlds 80% of the people rely on traditional medicine for primary health care. India and China stand premier to use folk / traditional medicine at their fullest.[1]

Plants in the World

Tribal societies throughout the world have their respective system of medicines, using locally available plant as ingredient of medicine. The revival of interest on natural drugs in health care through herbs and traditional methods as “herbal renaissance” is blooming across the world.

The efficacy of plant drugs and plant derived wonder drugs with miraculous therapeutic effects and rich ethno-pharmaceutical attributes are known from the tribes.[2]

Herbs in India

India is perhaps the largest producer of medicinal herbs and is rightly called the “Botanical garden of the world”. India has 45,000 different plant species, of these about 15,000 – 20,000 plants have medicinal values. However only about 7000 –

7500 plant species are used for their therapeutic value by traditional communities. Medicinal herbs have been in use for thousands of years, in one form or another under the indigenous system of medicine like Ayurvedha, Sidha and Unani.[3]

Indian subcontinent is enriched by a variety of flora-both aromatic and medicinal plants. This is due to the wide diversity of climatic condition in India ranging from deserts to swamp lands.[2]

Herbs in Modern Medicine

Herbs have been the main source of medicine throughout human history. That they all still widely used today is not a throwback to the dark ages but an indication that herbs are a growing part of modern, high-tech medicine. About 25-30% of today's prescription drugs contain chemicals derived from plants.

Some 119 chemical substances from 91 plants are now used in Western medicine. Of these 74% were folk medicines brought to our pharmacies through scientific research.

Researchers today examine folk or historical uses of plants to find new drugs for cancer, AIDS and even the common cold[4].

Role of WHO in Herbal Medicine

Two decades ago, WHO referred to traditional health systems (including herbal medicine) as "holistic" – that of viewing man in his totality within a wide ecological

spectrum and of emphasizing that ill health or disease in thought about by an imbalance or disequilibrium of man in his total ecological system and not only by causative agent and pathogenic evolution, probably implying that the indigenous system drugs (including herbal medicines) restore the imbalance leading to the cure of ill health (or) disease.[5]

Natural products in new drug discovery

Development of traditional herbal medicine into a modern drug of great therapeutic value is exemplified by the discovery of 'vinblastine' the treatment of leukemia in infants from the periwinkle in definitely a strong weapon to fight the cancer.

The pacific yew (Taxus brevifolia) also produced an anti-cancer drug 'Taxol'. A part from these researchers have started exploring the sea to obtain the novel drugs from the marine plants and animals like algae etc.[6]

Natural product will continue to be important in three areas of drug discovery.

- As targets for production by biotechnology.
- As a source of new lead compounds of novel chemical structures and.
- As the active ingredients of useful treatments derived from traditional system of medicine.[7]

Priorities in Medicinal plant research

The plant kingdom represents as enormous reservoir of biologically active molecules and sofar only a small fraction of plants with medicinal activity has been assayed. Nearly 50% drugs used in medicine are of plant origin.

There is therefore much current research devoted to the phytochemical

investigation of higher plants which have ethnobotanical information associated with them.

The phytochemical isolated are then screened for different type of biological activity.

The crude plant extracts can be first assayed for particular activities and the active fraction then assayed phytochemically.[8]

It is desirable to have a “need based” approach to research on medicinal plants including screening of plants for biological activity. This was the objective of a W.H.O regional group which met in 1980. research efforts could thus be directed for a number of diseases for which suitable drugs are not available in the modern system of medicine and where herbal drugs have a possibility of offering new drugs, some such conditions include the following where new drugs are urgently needed and the medicinal plant have already provided some leads :

Antiprotozoal drugs from plants, Antiulcer drugs, Antirheumatic, Antidiabetic, Anti asthmatic, Antiviral, hepatoprotective, Fertility control, Anti cancer, Urinary stones, Sedative and Tranquillizers and Laxatives.[9]

Research interest for many infections & infestations are not much focused by researcher. One among those conditions is for head lice infestation and the fungal infection dandruff etc. The use of herbals in these conditions is much to be explored.

Head Infestation

- Throughout history, human beings in all parts of the world have suffered from parasitical infestations including infestation of the hair and scalp by head lice

(Pediculus humanus capitis).

- Many methods of treating such head louse infestation have been proposed and utilized for centuries including, for example washing the head with strong soaps and lye and the utilization of kerosene to kill adult lice and their ova[10].

History about Lice

- These are worldwide in distribution being more common in the temperate climates. Infestation with lice were common during the world war which caused dislocation of population with over crowding of large communities in the most unsanitary and primitive condition.
- Even today lice occur in unhygienic individuals living in poor condition like the urban slums of the underdeveloped countries.
- Transmission of infection occurs by close body contact as for instance to children sleeping with infected parents in same bed.
- There are three types of lice.
    1. Pediculus humans var corporis (or body louse).
    2. Pediculus humans var capitis (Pediculus capitis)
    3. Phthrius pubis (or) pubic louse.[11]
- Adult males and females live in contact of the human body surface and feed by sucking blood. Unlike mosquitoes both the sexes feed on blood[11].

Lice Burden:-

Lice Infestation are common, found world wide and affect between 6 to 20 Million people every year it is very common and mainly affect children 3 & 12 years

old.[12]

The majority of infection are asymptomatic when symptoms are noted they may include a tickling feeling of something moving in the hair, itching caused by the an allergic reaction to louse saliva and irritability. Secondary bacterial infection may be a complication[13].

Head lice live in the hair on the head. But body lice a larger variety which live in clothing. Head lice as a species, go back millions of years, while body lice are more recent arrival[14].

Current treatment of head lice

- The various ectoparasiticides have been incorporated into creams, gels, shampoos, and the like for application to human hair and scalp.
- Some of the ectoparasiticides which have been utilised as pediculicidal treatments, since the early 1950s and are commercially available today in various forms include the insecticides like
- lindane and malathion as well as pyrethrin.

The treatment of head lice is now complicated by the emergence of resistance to pediculicides. Most clinical trials were done before resistance emerged and reviews of these trials do not give clear guidelines to the clinician[15].

The control of human head lice world wide depends primarily on the continued applications of organochlorine (DDT and lindane), organophosphorus (Malathion), carbamate (Carbaryl), pyrethrin, pyrethroid (permethrin & δ - phenothrin) and avermectin, insecticides. Their repeated use has often resulted in the development of

resistance, and increasing levels of resistance to the most commonly used pediculicides have caused multiple and excessive treatments, fostering serious human health concerns. These problems have highlighted the need for the development of selective p.humanus capitis control alternatives.

Natural products have been used in traditional medicine for thousands of years and recently have been of increasing interest, since the cost are usually lower and they are considered less toxic by the public.

Here the present a review on plant compounds used for the treatment of head lice.[16]

Some natural products used for treatment of head lice are[17,18]:

- *Neem (bark of Azadiracta indica)*
- *Annona Squamosa (seed extract)*
- Eugenea caryophyllus

Dandruff:

Dandruff is a major cosmetic problem that posseses very great public health concern both in developed and developing countries. The problem manifests as profuse white to silvery powdery scales in the scalp region often with moderate to severe itching.

Causative organism:

Pityrosporum ovale (Malassezia furfur), a yeast like lipophilic basidiomycetous fungus ,is considered to be the chief cause of the problem. Besides this, candida species is also suspected in the disease process of dandruff. These organism are

widely considered to be the commensal flora of the scalp and skin region.[19]

Global View :

A chronic inflammatory disease of the skin of unknown etiology.

Dandruff also refered as (Pityriasis simplex) is a common embarrassing disorder, which affects 5% of the global population.

Dandruff mostly occurs after puberty (between ages 20 – 30 Years), and affects males more than females.

The yeast Pityrosporum ovale is the causative microorganism of dandruff.

Pityrosporum ovale on the dermal liquids & protiens and facilitates lipase activity, which releases proinflammatory free fatty acids (FFAS) causing dermal inflammation and tissue damage.

The lipase activity indicates that in addition to hypersensitivity, Pityrosporum ovale releases toxic chemicals which contribute to the development of fungi infection.[20]

Types of dandruff

There are two common forms of dandruff. Dry dandruff and Greasy dandruff. The principle signs of dry dandruff are white scales on the hair and scalp accompanied by itching.

Greasy or waxy dandruff is characterized by scaliness of the epidermis, mixed with oils which causes it to stick to the scalp in patches. Dandruff may be associated with a dry or oily condition. Common causes included poor blood circulation to the

scalp, poor diet, improper hygiene, excessive use of styling products, dry indoor air, light fitting hats or infection. Long – term neglect of dandruff can cause baldness[21].

Symptoms of Dandruff :

Dandruff is characterized by scaling of the scalp, and is frequently associated with seborrhea which is the precursor of seborrhic dermatitis.

Dandruff is commonly aggravated by changes in humidity trauma (Scratching) season and emotional stress. Dandruff may improves in summer (as ultra violet rays from Sun light counteracts P.ovale) and may get worse in winter.

Other commonly associated symptom of dandruff are itching with scalp soreness. Seborrhic eczema is a more severe form of dandruff.[22]

TREATMENT OPTIONS

Dandruff shampoo

Choose an anti-dandruff shampoo that contain coal tar, salicylic acid, pyrithione zinc, sulfur or selenium sulfide, says Dr. Patnicia Farris walters, each ingredient reduces dandruff in different way.

Coal – tar based shampoo	:	Slow cell production
Salicylic acid based shampoo	:	slough off dead cells before they clump.

And both shampoos have anti-fungal properties and help fight invading yeast microbes, which is one of dandruff's most persistent triggers.

Currently available treatment options for the management of dandruff include therapeutic use of zinc pyrithione, salicylic acid immidazole derivatives, glycolic acid.

However these agents have certain limitations either due to poor clinical efficacy or due to compliance issues. Further more these drugs are unable to prevent recurrence which is the commonest problem[20].

Plants having anti-dandruff activity[23]

- Adiantum capillus – veneris
- Citrus hystrix
- Datura stramonium
- Gledissia australis
- Melaleuca leucadendron
- Petroselinium crispum

An integrated treatment to cure lice and dandruff

It should be noticed that there is no drugs is available to treat lice and dandruff both are very great public health concern. Moreover the drugs used may affects the eyes during their application.

So it is necessary to screen a drug especially of herbal origin to treat the both head lice and dandruff without affecting eyes.

Review of literature reveals that, the plant **Dichrostachys cinerea** belongs to the family Mimosaceae is used for the treatment of ophthalmia.

Reason for the selection of plant D.cinerea

It was also observed that the leaf paste of **Dichrostachys cinerea** is applied to the head to treat lice and dandruff by some villages of Tamilnadu. The enquiry and

discussion with some siddha practitioner confirmed the traditional usage of the **D. cinerea** leaf paste as antilice and antidandruff application. This prompted us to carry out the preliminary study to provide scientific basis for its antilice and antidandruff activity without ocular side effects so that to derive a lead molecule of plant based drug.

Chapter - II

REVIEW OF LITERATURE

General

Ethnomedical uses

- Information on the following plants used in the treatment of sveta (leucorrhoea) and rakta pradar (menorrhagia) gathered from the Dandakaranya is given: Abrus precatorius, Asparagus procumbens, **D.cinerea**, Erythrina indica, Ficus bengalensis, Hibiscus rosasinensis, Mimosa pudica and Phoenix sylvestris in pharmacognacy (Hemadrik, et al)[24].
- **D.cinerea** has 8 Subspecies some of which have been studied for their medicinal properties.
- The extract (2-Spoonfull) of **D. cinerea** is used as a purgative. The powder applied on gums for toothache, it is mixed with water and then taken orally for vermifuge[25].

Pharmacognosy

- It was reported that the **D. cinerea** supplementation significantly increased daily weight gain in cattle. The weight gain was the same for daily or weekly supplementation and could not be offset by compensatory gain in the control group. The defanating ability of **D. cinerea** played a significant role in increasing weight gain.
- So it was used as a browse plant for alternative source of crude protein. (Chango k. et al)[26].

Pharmacology

- Twenty-six plants used by Zulu healer for the treatment of pain and inflammation were assayed for cyclooxygenase inhibitory activity. The ethanolic extract of **D. cinerea** plant showed higher activity than the Aqueous extracts. (Mc Gaw L.J. et al)[27].

Aerial part

Pharmacology

- Antibacterial and Antifungal activities of n-hexane and chloroform (5 -10mg/ml) extracts of aerial part of **D. cinerea** were shown against E-Coli, P. aeruginosa, staphylococcus aureus and two fungi – Asperigillus flavus and Mucor-sp in nutrient agar and SDA mediums respectively by streak method. Antifungal activity was observed at high concentrations. (Pichai R.P et al)[28].

Leaves

Ethnomedical information

- It was reported that the leaves for **D. cinerea** are a laxative and used to treat gonorrhoea and boils. It is also used as a fodder. Powder from leaves is used in the massage of fractures. The leaf also used as pain –killers, oedema, gout, veneral diseases, swellings, diuretics and also naso-pharyngeal affections. (Burkill, H.M.) [29,30,31]

Pharmacognosy

- Severe defoliation as occur in insects outbreaks can alter the chemistry and food value of woody – plant leaves for insects for several years after defoliation

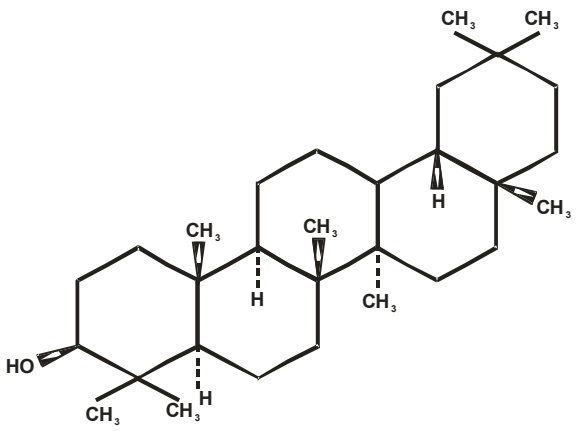
ceases by two hypothesis (AD and CNB).

- Manual defoliation of the three fast growing deciduous species were studied (Grewia flaveseens, Acacia torlilis, **D. cinerea**) resulted in increased resistance to insect attack and nitrogen and phosphorus concentration in leaves decreased and leaf total phenol and condensed tannin concentration increased. (Bryant J.P et al)[32].

Phytochemistry

- It was revealed that the leaves of **D. cinerea** contains hentriacontanol and β-amyrin.(Rastogi et al)[33].

β- amyrin



- It was found that **D. cinerea** had low dry matter degradation (DMD) and tannin content with a (CP) crude protein value of 13.77% which indicates that the CP may largely be un-available to animal. So it is used as a nutrition to animal. (Aganga, A.A et al)[34].

- The Sb, Cr, Ni, As, Pb and Cu contents in leaves of **D. cinerea** were significantly lower during dry season than during the rainy season, particularly in polluted site. Pb, Cu ($P<0.05$) content was higher than Arsenic ($P<0.05$). (Bako S. P. et al)[35].
- Chemical constituents as well as different fractions of protein and carbohydrate of mature leaves and twigs from 9 browse trees and 12 shrub were estimated. The shrubs like Acacia Catechu, Seurinega virosa, **D. cinerea** provide higher quantity B protein fractions. (Bhadauria K.K.S. et al)[36].

Pharmacology

- More effective antibacterial activity of aqueous, methanol than chloroform extracts (45mg/ml 0.1 ml/cup) of leaves and fruits of **D.cinearea** were shown against Bacillus subtilis, staphylococcus, aureus, E-coli, Pseudomonos aeruginosa by cup- plate agar diffusion method and MIC was determined by agar dilution method. (Eisa M.M et al)[37].

Stem Bark

Ethnomedical information [38,39,40,41]

Tender shoots of the plant are bruised and applied to the eyes in case of ophthalmia. Bark to treat dysentry, toothache, elephantiasis, vermifuge, snake–bite, leprosy, syphilis, gonorrhoea, anthelmintic, purgative, laxative and diuretic, arthritis, abortifacients, and also used for pulmonary trouble, pain killers, (Burkill H.M, Varier's P.S., Krithikar, K.R.)[31,38,39,40,41]

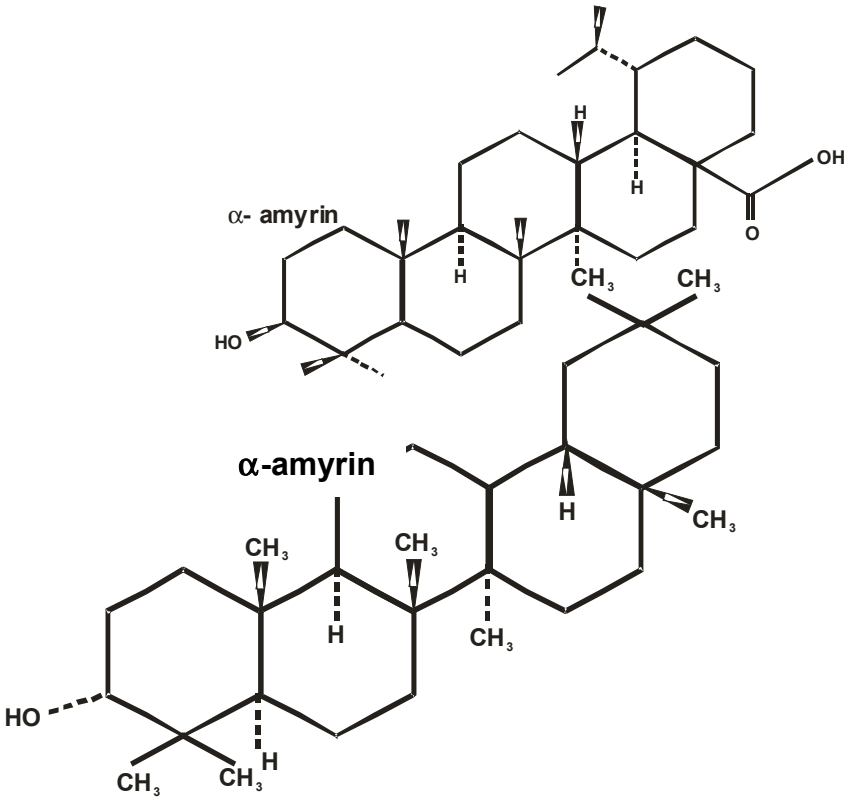
Pharmacognosy

- Fresh young bark from a stem 2 cms in diameter is about 2 to 3mm in thickness. As the stem increases in diameter the nature and colour of the outer surface of the bark change gradually. A transverse section of a fairly mature stem bark of **D. cinerea** shows a brown rhytidome, long wavy medullary rays, cork and phellogen, phelloderm, a few parenchymatous cells with small rhomboidal crystals of calcium oxalate, and starch grains. (Aiyer K. N. MA et al)[42].

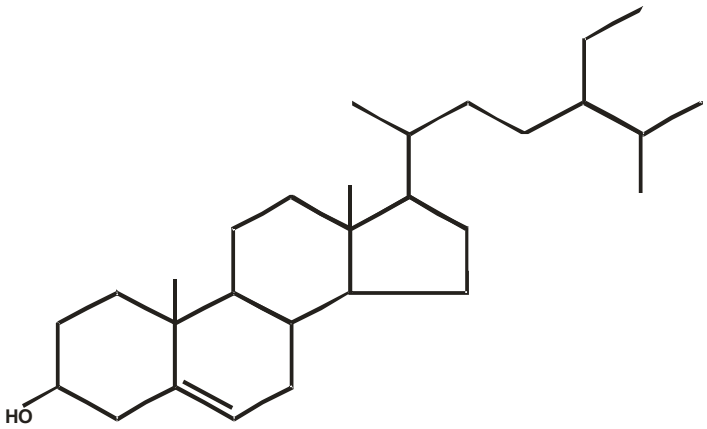
Phytochemistry

- It was reported that, the Betulinic acid isolated from the bark of **D. cinerea** were used a number of diseases. (Dr. Dukes)[43].
- Friedelin, Fridldelan - 3β-ol and α- amyrin also isolated from bark. (Rastogi R.P)[33].

Betulinic acid : acid



β-Sitosterol[46]



Pharmacology

- The antibacterial activities of ethylacetate, methanol and aqueous extracts of stem bark of **D. cinerea** have been calculated. A series of known compounds have been investigated in ethyl acetate, amongst which many are reported here for very first time. (Fotie.J, et al)[44].

Wood and Wood Powder

Ethnomedicinal information

- Branches are used for fencing and for cattle bomas[45].

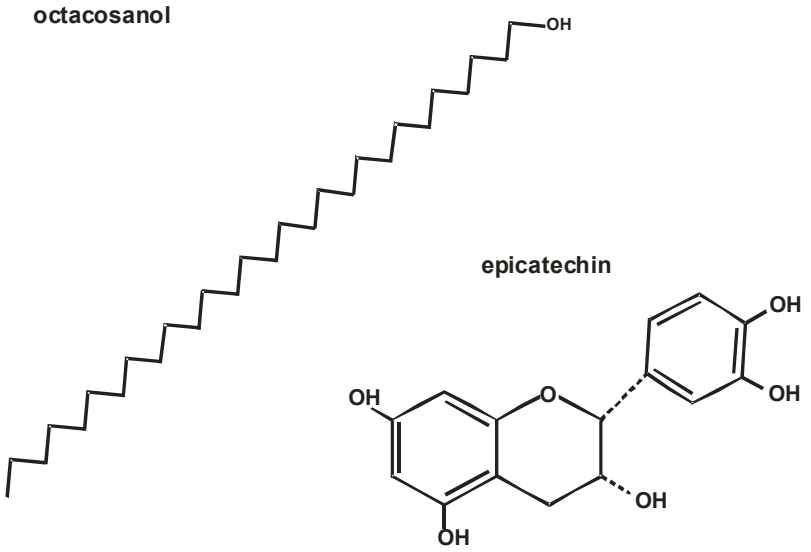
Pharmacognosy

- The wood is very heavy and hard, (fine dark brown heartwood) termite resistant, but usually of small dimensions. It is used for walking sticks and tent pegs.

Phytochemistry

- Heart wood of **D. cinerea** yielded octacosanol. (Rastogi et al)[33].
- The yield of (-) epicatechin is about 0.45% of the dried wood powder of **D.cinerea** by extract with different solvent Finally the pure (-) epicatechin was obtained by column eluted with chloroform and methanol. This compound known to posses several therapeutic activities (Madhu sudana R.J. et al)[47].

- A novel compound namely (-) mesquitol were isolated from the wood of **D. cinerea**. The yield of (-) mesquitol is about 1.5% by eluting the column with chloroform methanol gradient. The fraction eluted at 5% methanol in chloroform and contentrate it to get pure – mesquitol (Rao J.M et al)[48].



Pharmacology

- A novel compound namely (-) mesquitol were isolated from the wood of **D. cinerea**. It is a better antioxidant molecule than presently used probuccol and α-tocopherol. It may have better therapeutic potential in inflammatory disease condition, cancer, hepatotoxicity etc.
- The (-) epicatechin derived from a dried wood powder of **D. cinerea** were studied. The compound known to posses several therapecutic activities like anti-diabetic, inhibition of angiotension-I, Antiviral, and Inhibition of HIV-1. (Madhusudana R.J. et al)[47].

Fruits

A small, dry, dark brown glabrous compressed thick linear (or) strap – shaped coriaceous indehiscent or irregularly opening some what jointed or subarticulated pod 5 to 7.5 cms. long & 6 – 9mm thick. (Aiyer, et al.)[42]

Phytochemistry

Fruits of Acacia nilotica and **D. cinerea** were examined in invitro and invivo trials. Dry Matter intake, Dry Matter Degradation were lowest and N – retention negative in goats fed A. Nilotica as supplement, but highest in goats fed with **D. cinerea**. Supplementation with **D. cinerea** fruit resulted in improved goat performance. (Smith T. et al)[49].

Pharmacology

The antibacterial activities of ethylacetate, methanol and aqueous extracts of

stem bark of **D. cinerea** have been calculated. A series of known compounds have been investigated in ethyl acetate, amongst which many are reported here for very first time. (Fotie. J, et al)[44].

More effective antibacterial activity of aqueous, methanol than chloroform extracts (45mg/ml 0.1 ml/cup) of leaves and fruits of **D. cinearea** were shown against Bacillus subtilis, staphylococcus, aureus, E-coli, Pseudomonos aeruginosa by cup-plate agar diffusion method and MIC was determined by agar dilution method. (Eisa M.M et al)[37].

Pods and Seeds

Ethnomedical information

Seed-Pod of **D. cinerea** was used as pain-killer and antidotes (Venomous stings, bites, etc). (Bryant J.P. et al)[32].

The pods form a valuable source of protein in Tanzania, and are highly valued for goat fodder in Dodoma [36,41].

Phytochemistry

The preferential and extensive browsing of this indehiscent legume is apparently not due to any exceptional nutritive values of the pods and seeds.

The strong rich aroma of the infructescence which initially attracts animals to this food sources the structure of the infructescence, ensures that upon location a large number of pods and seeds are consumed.

Ingestion of the pods will provide fibre, lipid, fatty acids, carbohydrate, amino

acids, nitrogen and protein (Van staden J et al)[50].

Seeds

Seeds of **D. cinerea** are ovate to obovoid compressed glabrous or naked[28].

Phytochemistry

Fatty acid composition of seed oils of eight species viz Cleome viscose, Barleria acarnthoides, Mimosa Hamata, Dichrostachys cinerea, Argyreia nervosa, Clitoria ternatea, Tephrosia purpurea and Sesamum mulayanum are reported. All the seeds were collected from arid zone of Rajasthan and examined for their physico-chemical characterization and fatty acid composition were compared. (Deora. M.A. et al)[51].

Roots

Ethnomedical information

The roots are bitter, astringent, acrid, thermogenic, anti-inflammatory, anodyne, digestive, constipating, lithontriptic and diuretic, and are useful in vitiated conditions of Kapha and Vata, inflammation, arthralgia, elephantiasis, dyspepsia, diarrhoea, vesical calculi, nephropathy and pain in the joints[39,40,51].

The root infusions are taken for leprosy, syphils coughs, as an anthelmintic, purgative. The roots are chewed and placed on the sites of snakebites and scorpion strings.

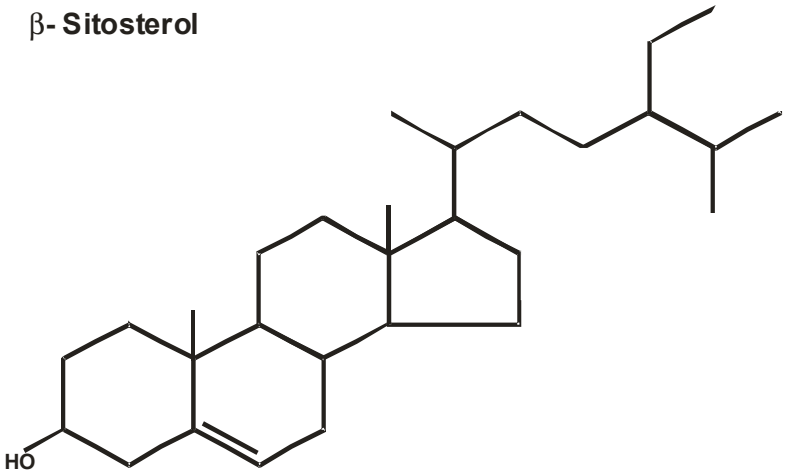
Pharmacognosy

Ash value, acid insoluble ash, alcohol and water soluble extractives, crude fibre contents, fluorescence analysis, TLC pattern of pet-ether extract and microscopical studies of the root have been determined. (Dennis T.J. et al)[53].

Phytochemistry

It was reported that n-octacosanol, β - amyrin, friedelan – 3-one, friedelan – 3 β01 and β-sitosterol from roots, of **D. cinerea**.

β- **Sitosterol**



Pharmacology

- More effective antisnake venom activity of methanol extracts of roots of **D. cinerea** than etherial and aqueous extract were studied against Russell's viper venom when administered after 1 minute after the venom by invivo method. The best protection was obtained with 200mg/kg. (Mishal H.B)[54].
- The oral administration of methanolic extract of root of **D. cinerea**, Acacia nilotica Cassia abbreviata, Solanum incanum, Vernonia amygdalina and Zanha

africana were most frequently used plants for the treatment of sexually transmitted diseases. (Kambiz: L et al)[55].

Biological activity

The antibacterial activity of tannin isolated from the Root of **D. cinerea** were shown against staphylococcus, aureus, Sh. boydii and Sh. Flexneri, E. Coli and P. aeruginosa by agar diffusion method the MIC was determined.

The increased concentration of tannin were shown effective antibacterial and bactericidal actions. (Banso.A et al)[56]

Various formulations in which D. cinerea as one of the ingredient for the treatment of various diseases world wide[57]

Leaves

Receipes	Region	Diseases
Leaves of **D. cinerea** roots of Solanum aculeastrum	East Africa	Epilepsie
Leaves Maceration	South Africa	Eye medicine
Rub with the leaves of **D. cinerea**	West Africa	Neuralgia stiff neck
Young leaves roasted, pounded in to a fine powder	West Africa	Wound
Young leaves ground into powder mixed with palm kernel oil	West Africa	Burn
infusion leaves	East Africa	Hernia
Decoction (or) maceration of leaves and barks of **D. cinerea**	Central Africa	Snake bite

Receipes	Region	Diseases
Pounded leaves	West Africa	Antiseptic
Pounded leaves	East Africa	Gonorhoea, Anesthetics
Leaves **D. cinerea,** Markhamia Lutea extract with water 1 glass in the morning	Central Africa	Female, Uretal leak
3 teaspoon full of powdered leaves is mixed with 1 cup water mixture applied externally	East Africa	Pneumonia
Fresh or dried leaves are crushed 1 x /day for 3 days	East Africa	Astringent on the Skin
Pounded leaves	Central Africa	Anthelmintic

Bark

Receipes	Region	Diseases
Chip it and decoct to liquid	East Africa	Bellyaches, Veneral Diseases and for Abortion
Stems, chewed, juice VO	West Africa	Colic
Juice, 3 drops in each ear, morning and evening until recovery	Central Africa	Otalgia, Otitis
Barks of **D. cinerea** pounding to boil. VO	East Africa	Sexual impotence & erectile dysfunction

Seed

Receipes	Region	Diseases
Seeds of **D. cinerea,** RNS, Unction	East Africa	Itch

Fruits

Ripe fruit of **D. cinerea** powder, local application	East Africa	Syphylis
Grounded fruit into powder and applied on penile sores	East Africa	Sexually transmitted diseases

Root

Receipes	Region	Diseases
Bulb of Gladiolus psittacinus barks of the underground part of D.glomerata, powder, to snuff	West Africa	Psychosis
grind 30g roots in powder, place in a hot plate, inhalation & 30 g powder in 1l. B. water, VO 1 tablespoon 2 x / day until healed	South Africa	Cough
Roots of **D. cinerea** decoction (water) local application	West Africa	abscess, Bronchitis, aphto, mouth wash
dried powdered roots added to tea	East Africa	heart pain
Roots of **D. cinerea** powder drops in nose	East Africa	Bleeding at the nose
Fresh roots chewed and pasted to the bite	East Africa	Snake
Roots of **D.cinerea**, local application scarification	East Africa	Rheumatism

HEAD LICE

The life cycle of pediculus humanus var capitis has been fully reviewed.[58]

Various treatment methods using drugs like lindane, pyrethrins, permethrin, melathione etc have been discussed in detail (Dirk M.)[59]

The nature of head lice **(P.capitis)** transmission was studied and it was suggested that head lice are most likely to rely on head to head contact for transmission. (Canyon D.V. et al, 2002).[60]

Plants screened for Antilice Activity

Five essential oils and 9 of their components were compared to Diethyl toluamide (DEET) for their repellent activity against the human body louse (P.humanus humanus). It was revealed that citronella was the most potent repellant for lice followed by citronellal, Rosemary, Geraniol and DEET the differences however were not significant (Mumcuoglu et al).[61]

An attempt was made to provide a concise compilation of the available information on antilice activity of plant extracts and plant derived compounds. In a filter paper contact bioassay, a 0.125 mg/cm2 pediculocidal activity of cardamom Ceylon, clove bud, myrtle, rosewood and sage oils was comparable with that of the test insecticides.

In fumigation test at 0.25mg/cm^2 Eucalyptus, margeron, penny royal and rosemary oils were more effective in closed containers than in open ones, indicating

its action in the vapour phase.

The toxicity of Eugenia caryophyllata bud and leaf oil derived compounds (acetyl eugenol, β-Caryophyllene) against eggs and females of pediculus capitis was examined using direct contact application and fumication methods and compared with those of the widely used phenothrin and pyrethrum by filter paper diffusion bio assay (Yang Y.C. et al).[62]

An overview was given on published evidence and the use of natural products pediculicides and ovicides in clinical trails and invitro studies, and insist on urgent need to increase research assessing the effectiveness and safety of promising compounds were made (Heukelbech J. et al).[16]

Antilice screening methods

The modified protocol for determining resistance in body lice P.humanus humanus was proposed to make a holding period of lice shorter and handling of lice easier (Seichner B.C, 1999).[63]

A research to demonstrate to rear head lice successfully invitro through a complete life cycle using 4 geographically distinct colonies of the human head lice **P.humanus capitis** was demonstrated (Lee M.T. et al).[64]

Automated mass rearing system for maintaining fluid sucking ectoparasites, including human head louse invitro to study toxicological, behavioral and diseased transmission investigation was demonstrated (Lee M.T et al).[65]

The susceptibility of head louse eggs to insecticide spinosad (a macro cyclic lactone) was assessed by immersion method showing similar susceptibility in early medium and late stages of head lice eggs were studied (Cueto GM et al).[66]

An attempt was made to access the contribution of the components of essential oils, **monoterpenoids** were tested in invirtro toxicity model against both human lice and their eggs at different concentration and shown that + - **terpinen – 4-ol** was the most effective compound against lice whereas **nerolidol** was particularly lethal to eggs but ineffective against lice (Priestley C.M. et al).[67]

Dandruff and screening method for dandruff

The antidandruff action of **'Anti – dandruff hair oil'** having **Hibiscus rosasinensis, Embilica officinalis, Centella asiatica, Eclipta alba** was demonstrated. It was also suggested that the action must have be due to the synergistic antifungal, antiinflammatry and local immuno-stimulatry action of its ingredients (Vijayanthi G. et al).[22]

The excellent anti dandruff action of "Anti – dandruff shampoo" having **Rosemarinus officinalis, Vetiveria zizanioids, Citrus limon, Nigella sativa, Santalum album, Melaleuca alternifolia** and **Melaleuca leucodendron, Ficus bengalensis** as ingredients was recommended as safe management of dandruff (Ravichandra et al).[20]

Dano, a poly-herbal hair oil was studied for anti-dandruff activity using microbiological and clinical lists. There was is clear symptomatic relief from dandruff in

all the volunteers after 10 days of use (Krishnamoorthy JR et al).[19]

A modified medium for the culturing of **Malassezia furfur** has been proposed and also growth was determined in the presence of different carbon sources, temp, p^H and salinity (Vijayakumar R et al).[69]

Chapter - III

AIM AND OBJECTIVE

The head lice, **Pediculus humanus capitis** (Phthiraptera: Pediculidae), is an obligate ectoparasite of human that causes pediculosis capitis, a nuisance for millions of people world wide, with high prevalence in children. Pediculosis capitis has been treated by methods that include the physical remotion of lice, various domestic treatments and conventional insecticides. None of these methods render complete protection, and there in clear evidence for the evolution of resistance and cross-resistance to conventional insecticides[57].

Current Anti-lice treatments.

There are several types of treatments in the market.

Chemical treatments[16,67]

These conventional treatments contain different insecticide substances.

- Pyretherin and permethrin
- Malathion
- Lindane
- Piperonyl

All of these pediculicidal agents act efficiently against the adult head lice and less efficiently against nits. However, some of them are neurotoxic. Since they are not selective, they act on nervous system of insects as well on that of higher life forms.

Other elements strengthen their toxic effects[16]

They are only midely effective in eliminating eggs before they hatch, thus requiring repeated treatment applications. They must be applied on a rather permeable are (the scalp), in a population mainly composed by children whose less developed immune system and detoxification process makes them most sensitive to insecticides.

The continued use of these products induces resistance. Consequently, higher concentration or longer treatments are required, thus increasing the risk for adverse effects.

Lindane, an organochlorine pesticide, is the most toxic of them and it is currently forbidden, or in process of becoming forbidden in many countries. The main absorption ways are the oral, dermal and the respiratory system. Because of its high liposolubility, it is stored in the adipose tissue and other fat-rich tissue such as liver, kidneys and nervous system. Symptoms resulting from the exposition to lindane resemble DDT intoxication.

Malathion is an organophosphorus insecticide, very toxic by ingestion, irritant by skin or eyes contact and probably mutagen Pyrethrisis: are natural insecticides produced by the species chrysanthemum cinaerefolium used as a domestic insecticide because of their high insect-killing power and low toxicity for mammals. However, they undergo photolysis when exposed to sunlight and air and degrade yielding inactive products. To cope with these stability problem, derived substances such as

permethrin were synthesized.

Permethrin is recommended as a first choice treatment, although recent studies report decreased sensitivity and increased resistance of lice of these products.

Pyrethrum and Permethrin are considered the safest products because of the low absorption occurring at the concentration levels present in most of commercial formulas[67].

An alternative to pesticide treatments, in the majority of situations, a carrier oil has sufficient suffocating capabilities to make almost any mixture of essential and carrier oil effective in killing lice and possible effectiveness of penetration using lipid based compounds[68].

Non-toxic alternative options are hence needed for head lice treatment and /or prevention and natural products from the plants.

The another skin condition affecting the scalp are dandruff which consists the excessive making of the skin as the consequents of accelerated sheding of epidermal cells, large clumps of horny cells of head are produce. It is a common embarrassing disorder.

Currently available treatment options have various limitations either due to poor clinical efficacy are due to the compliance issue and unable to prevent recurrence. So far no herbal treatment available to treat the both the problems of head namely lice and dandruff. If was observed that leaf paste application of **D. cinerea** (L.) W&Arn is

used for lice infestation by villagers, in some areas of Tamilnadu and discussion with traditional practitioners confirmed its uses for lice treatment.

Review of literature reveals the presence of anti fungal activities of its extract[24]. It prompted us to investigate the scientific basis of its traditional use for the treatment of lice. We have also planned to assess its antidandruff activity also considering its antifungal activity. So that a single medicinal plant drug which is widely available, to treat both infection of head. The available literature reveals that no pharmacognostical, anti lice, anti dandruff studies have been carried out.

Aim

To carry out pharmacognostic, preliminary phytochemical studies and to perform an integrated invitro preliminary screening against lice and the fungus **Malassezia furfur**, for the ethanolic extract of the leaves of **D.cinerea** (L.)W& Arn.

Objective

The objective of the study was divided into three parts:

Part 1 : Pharmacognostic studies

- Collection and authentication of the leaves of **D.cinerea.**
- Detailed pharmacognostic study on leaves of **D.cinerea** to obtain the best possible structural detail to assist in the solution of taxonomic problem, to avoid misleading of diagnostic features by over simplified description along with its

stem. So that to identify the stem in the leaf powder and to check the presence of any specific character pertaining to them which will support the analysis of pure leaf powder.

Moreover to carryout quantitative microscopy and other parameters.

Part II – Phytochemical Studies

- Preliminary phytochemcial studies on the crude powder and on the different extracts of the leaves of **D.cinerea**.
- To perform thin layer chromatography with various solvents, of ethanolic extract.
- Analysis of ethanolic extract of the leaves by HPTLC.
- To carry out column chromatography to separate phytoconstituents and biologically active fractions.

Part III- Pharmacological Activity

To evaluate (i) the ethanolic extract, (ii)isolated fractions, (iii)fixed oils treated with powder and ethanolic extract and (iv) aqueous paste for in vitro preliminary anti lice activity. A parallel evaluation of *in vitro* anti dandruff activity by disc diffusion method, using ethanolic extract of **D.cinerea**.

Chapter - IV

PHARMACOGNOSTIC STUDIES

SECTION - A

MACROSCOPICAL STUDY OF Dichrostachys cinerea (L) W& Arn[35,39,70]

D. cinerea Wt & Arn is a profusely branched thorny shrub or small tree upto 2m in height, branches and branchlets armed with spiny tips; bark grey or light brown coloured, furrowed: leaves bipinnate belonging to the family Mimosaceae.

Plant Taxonomy (Scientific Classification)[72]

Kingdom	:	plantae - plants
Subkingdom	:	Tracheobionta – vascular plants.
Phyllum/division	:	Angiospermae
Super division	:	Spermatophyta – seed plants
Class	:	Magnoliopsida (Dicotyledonous)
Sub Class	:	Rosidae
Order	:	Fabales
Family	:	Fabaceae
Sub family	:	Mimosoidae
Genus	:	Dichrostachys (DC), Wight & Arn
Species	:	Dichrostachys cinerea (L) Wight & Arn
Synonym	:	Dichrostachys glomerata (Forssk)

Dichrostachys nutans (Pers) Benth
Dichrostachys nyassana Taub,
Dichrostachys arborea N. E. Br
Mimosa Cinerea L
Mimosa nutans (Pers)
Cailliea Dichrostachys Gvill & Perrott

Common Names[103] : el Marabu (Cuba), Kalahari – Weihnochtsbaum (German), Tamil – ÅPRûX chinese lantern (E), mupangara (S), muruka (S) sinckle bush (E), ugagu (N)

Vernacular Names

African	:	Sekelbos
Burma	:	Sitbyu
Ceylon	:	Vidattal
English	:	Sickle bush
Gujerati	:	Marudi
Hindi	:	Vurttuli, Kheri
Kannada	:	Odatare
Malayalam	:	Vitattal
Marathi	:	Segum – Kati
Panch mahals:		Vellantaro
Sanskrit	:	Vellantarah

Tamil : Vidattalai, Vidatter, Varittula

Telugu : Veluturu, Venuturu

The generic name **'Dichrostachys'** means '2 coloured spike' and **cinerea** refers to the greyish hairs of typical subspecies, which confined to India from the Greek 'Konis' & the Latin **'cinerea'**.

Geographical Distribution[29,38]

D. cinerea is a much – branched thorny shrub sometimes a small tree upto 2m in height, bark light coloured furrowed branchlets ending in spines, indigenous to N.W – India, central India Rajputana, Ceylon, Malay – Islands, N. Australia.

Native

Cameroon, Ethiopia, Kenya, Ghana, Madagascar, Nigeria, Somalia, South Africa, Sudan, Uganda, Zambia, Swaziland, Tanzania.

Exotic

Australia, Botswana, Benin, Brunei, Cambodia, Burkina Faso, Central African Republic, Congo, Iran, Indonesia, Malaysia, India, Republic of Zimbabwe.

Habit : Shrub

Habit and Habitat[25,34,35,55]

The plant **D. cinerea** is distributed throughout the dry and warm parts of India. It commonly grows on stony hills and in the dry scrub forests of the plains. It is occasionally cultivated. **D. cinerea** penetrates clear cut areas far into the rainforest

zone. In Malaysia it occurs in areas with strong seasonal climate usually on poor, occasionally clayey soils, in brush wood, thickets, hedges, teak forest and grassland. Forms dense hammocks on lateritic soils in Senegal and sudan, while in India it occurs in dry deciduous forest. It can be an indicator of overgrazing in low rainfall areas. Usually not frost resistant and tolerance is less on poor soils, but definitely drought resistant. It is fire resistant and does not tolerate water logging (plate no-1, Fig -1).

Description

Leaves

Colour : Green

Odour : distinct agreeable Odour

Taste : Bitter

Texture : Soft

Margin : Entire

Alternate, bipinnate, the main rachis more or less softly pubescent, with a small erect gland between each pair of pinnae: stipules – 4.5mm long, subulate from a triangular base pinnae 8-14 pairs, 1-1.6 cm long. (Plate No-2. Fig No -1).

Leaflets

Minute, sessile 12-20 pairs, close, linear or strap shaped oblique, subacute (Plate No – 3).

Bark

Grey or light brown coloured, furrowed with many dark black spots.

Inflorescence

It arise singly or pairs from short, axillary branchlets or in the axile of leaves, they are short peduncled dense flowered drooping axillary spikes 2.5-5cm long, solitary or apparently fascicled (Plate No -4).

Two types of flower are present namely bisexual and neuter.

Bisexual - Yellowish color, and formed distal half of the flower.

Neuter - Pinkish color and formed basal half of the spike.

Flowers

The most unusual aspects of the tree is the attractive flowers. Flowers are numerous, crowded in dense axillary or extraaxillary spikes 2.5-3.8cm long. The upper half of the spike yellow, the lower red.

Stamens 10, of the perfect flowers in the upper half of the spike yellow.

Staminoides in the lower half of the spike 1.3cm long, much longer than the stamen, red.

Calyx

0.8mm long, membranous, shortly toothed.

Corolla

2.25cm long. (about 3 times as long as calyx)

Petals

5, Valvate, connate below.

Ovary

Subsessile, many ovuled with a filiform style ending in a terminal truncate or capitate stigma.

Fruit

A small, dry, dark brown glabrous compressed thick linear (or strap-shaped coriaceous idehiscent or irregularly opening some what jointed or subarticulated pod.

Pod

5 - 7.5 by 0.6-1cm, glabrous, flat, subarticulated, dark brown, twisted up when ripe, and containing 6-10 seeds there being no septa or partition between seeds. (Plate No - 5)

Seed

Obovate, compressed, 4-6 and 3-4.5, deep brown, glassy appearance. (Plate No – 5)

Flowering

The plant is in flower from April onwards till about October.

Fruiting

Fruits ripen in March – May.

Biophysical limits[25]

Altitude	:	Upto 2000m
Annual Temperature	:	2-50^{o}c
Annual Rainfall	:	200-400mm

Soil type : Sandy loamy soils, it can tolerate all a wide pH range

Life cycle stages[29]

Seeds survive long in the soil. The growth of the plants is very fast. Young plants may produce seeds. Adult plants can survive a very long time, producing seeds almost all year long (Fournet J).

Mean of propagation[37,104]

Seedlings, direct sowing, and root suckers. it is easily established from root or stem cuttings.

Temperature : Occurs in frost-free localities

Water : Occurs in medium rainfall areas

Crop management : It is fire tolerant

Seed collection and storage

As soon as the pods ripen on the tree, they should be collected and the seeds taken out, dried and stored. About 1million seeds/year are expected from a plant and most of the seeds are viable.

Seed biology of D. cinerea

No. of Seeds/kg	Germination %	Time taken for germination (in days)
39,000	75%	15-20 days

Pretreatment

Scarified seeds give better germination, and a pretreatment of 25 minutes in concentrated sulphuric acid gives optimum germination of 3-7 days for freshly collected seeds.

Reproductive Biology[29]

In Indonesia, **D.cinerea** has been found flowering from September to June and fruiting from March to May, sporadically in other months in Southern Africa flowering is from October to February and fruiting from May to September. The structure of the inflorescences suggests pollination by bats. The infructescence has a strong aroma, which probably attracts animals to feed on the pods. A fraction of seeds exhibit polyembryony with usually 2, sometimes 3 or rarely more embryos.

SECTION – B

MICROSCOPICAL STUDIES[73,74,75,76,77,78]

DICHROSTACHYS CINEREA (L) Wight & Arn

Collection of Specimens

The plant specimens were collected from Forest of Perambalur District Tamil Nadu during August 2007. Care was taken to select healthy plants and for normal organs. The leaves were cut and removed from the plant and fixed in FAA (Formalin-5 ml + Acetic acid – 5 ml + 70% ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^{0}c), until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. After dewaxing the sections were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cyto chemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., Where ever necessary, section were also stained with safranin and fast-green and potassium iodide (for starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide and epidermal peeling by partial maceration employing jeffrey's maceration were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs where ever necessary. Photographs of different magnifications were taken with Nikon labphot 2 microscope unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

Leaflet

150 μm thick in the middle and 40 μm at the margin elliptical with narrow margin. (Plate No – 6, Fig No -2)

Epidermis

Thin **tanniniferous** and semi circular with raised outer periclinal walls (10 μm thick).

Mesophyll

Not well differentiated into palisade and spongy parenchyma. **8-10 layers of short, rectangular tanniniferous compact and darkly staining cells**.

Vascular bundle

Both midrib and lateral vein bundles are quite prominent without projecting much beyond the surface of the lamina. The midrib bundle is circular and 90μm in diameter.

Xylem occurs as **conical cluster** and **phloem as a thick** arc. It is encircled by a thick sclerenchymatous bundle sheath. The sub marginal vascular strands are smaller, similar to the midrib bundles. (Plate 6)

Stomata

Occur on the abaxial epidermis and paracytic (rubiaceous) type with **smaller** and another **larger** subsidary cells, which are parallel to the long axis of the guard cells.

The stomata are elliptical with wide slit like stomatal aperture. The **epidermal cells are amoeboid in shape** with wavy thin anticlinal walls.

The epidermal cells along the veins are **longitudinally elongated and straight walled.** (Plate No -7)

Venation pattern

The leaflet has a thick central midrib which extends upto the tip. The primary lateral veins are equally prominent and run obliquely forward. The secondary lateral veins are thin and less distinct.

The vein islets are not well defined. There are short, unbranched vein termination which are characterized by presence of one or more rectangular **sclereids**. There terminal **sclereids** have thick walls and circular, dot like simple pits.

Unicellular, **conical trichomes,**all seen along the margin.(Plate No.8)

Crystals

The mesophyll tissue and in the Lumen of the sclerenchymatous cells of vascular bundle randomly contains **calcium oxalate prismatic crystals**. They are cubical, **rhomboidal** and rectangular shape. (Plate 9)

Rachis

In cross-sectional view, the rachis is shield shaped with **two adaxial-lateral short wings,** shallow median concavity and wide adaxial side. It is 600 μm in vertical plane and 650μm in horizontal plane. The epidermal layer is thin and comprises of small thick walled cells. The ground tissue is **narrow and parenchymatous**. The cells are fairly wide, compact and thin walled. The vascular system consists of two

wing bundles. The wing bundles are circular with eccentrically thick, **sclerenchymatous bundle sheath enclosing a small group of xylem and phloem**.

The main vascular system has a thick, circular hallow sclerenchymatous cylinder which encloses five prominent vascular bundles. Of the five bundles, three are abaxial in position, one of them is mediate bundle, two are lateral bundles. The other two bundles are adaxial-lateral in position. The adaxial bundles are split into two units which are closely placed. The vascular bundles are collected with wide, thick walled cluster of vessels and thick bands of phloem.(Plate No-10, Fig No-3)

Stem

The stem is circular in sectional view measuring 1.6 mm decimeter. It consists of epidermis, cortex, sclerenchyma cylinder, vascular cylinder and wide pith. (Plate No. 11,12,13)

Epidermis

It is thin and continuous. The epidermal cells are normally elliptical, thickwalled and darkly staining. (Plate 11)

Cortex

It consists of about eight layers of tangentially elongated, thick walled, compact parenchyma cells. A row of cells lying in the median zone of the cortex dilate radially and divide by periclinical wall giving rise to the origin of the **first phellogen**. The cortical zone is 90μm wide. (Plate 12)

Vascular cylinder

It is thick hollow continuous cylinder of wavy outline. It has outer cylinder of secondary phloem with sieve elements and phloem parenchyma, and is ensheathed externally by a thick continuous cylinder of sclerenchyma cells. The sclerenchyma cells are of gelatinous fibres comprising of **outer lignified wall** and **inner gelatinous** (or) **mucilaginous wall.** (Plate 13)

Secondary xylem

It consists of wide (30-50μm), circular thin walled vessels which are mostly solitary and diffuse in distribution. The xylem fibres are thick walled, lignified and have wide lumen. Thin, straight xylem rays are seen. (Plate -13)

Pith

The pith is wide circular, thickwalled and compact parenchymatous cells. Some of the pith cells have **dark solid bodies.** (Plate – 13).

Powder microscopy of leaf powder of D.cinerea (L.) Wight & Arn.
Organnleptic Characters

Nature : Coarse

Color : Green

Odour : Distinct odour

Taste : Intensively bitter

The powder microscopy of the leaf powder of **D.cinerea** reveals the following

characters.

- Epidermal cells with stomata and trichome.
- Paracytic type of stomata
- Conical trichomes are seen.
- Palisade cells with epidermal cells.
- Crystals distributed in the mesophyll tissue.

SECTION – C

QUANTITATIVE MICROSCOPY FOR LEAVES OF *D. cinerea*[79,80,81,82,83,84]

Microscopic Schedules

The vein islet number, vein terminal number, stomatal number and stomatal index were determined on fresh leaves using standard procedures.

A. *Vein islet number and Vein terminal number*

The term vein islet in used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. The number of vein islets per sq. mm., area is called vein islet number.

Vein terminal number may be defined as the number of vein terminals present in one sq., mm. area of the photosynthetic tissue.

Determination of Vein Islet Number and Vein Termination Number

Leaf lets were cleared in chloral hydrate, stained and mounted on a slide.

A camera lucida is set up and by means of a stage micrometer the paper in divided into squares of $1mm^2$ using a 16mm objective. The stage micrometer is then replaced by the cleared preparation and the veins are traced in four contiguous squares, either in a square 2mm x 2mm (or) rectangle 1mm x 4 mm.

When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square (or) rectangle but included if cut by the other two sides.

Ten readings for vein islet and vein termination number were recorded.

Table - 1

VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER OF D. Cinerea

Observation number	Vein Islet Number	Vein Termination Number
1	12	8
2	11	6
3	14	6
4	12	9
5	10	6
6	12	7
7	13	9
8	12	8
9	15	7
10	14	8

Range	Minimum	Average	Maximum
Vien islet Number	10	12.5	15
Vien Termination Number	6	7.4	9

Stomatal number

Stomatal number is defined as the number of stoma present in one square mm area of the photosynthetic tissues.

Method

Using fresh leaves, replicas of leaf surface may be made which are satisfactory for the determination of stomatal number and stomatal index. An approximate 50% gelatin and water gel is liquified on a water – bath and smeared on a hot slide. The fresh leaf is added, the slide inverted and cooled under a tap and after about 15-30 min the specimen is stripped

off.

The imprint on the gelatin gives a clear outline of epidermal cells, stomata and trichomes.

Table - 2

STOMATAL NUMBER

Observation Number	Lower epidermis
1	78
2	80
3	81
4	82
5	79
6	84
7	83
8	75
9	80
10	77

Range	Minimum	Average	Maximum
Lower epidermis	75	79.9	84

B. Stomatal Index

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

I, Stomatal index = S/S+E x 100

Where S = Number of stomata per unit area

E= Number of epidermal cells in the same unit area

Determination of Stomatal Index

The procedure adopted in the determinations of stomatal number was observed under high power (45 x).The epidermal cells and the stomata were counted. From these values the

stomatal index was calculated using the above formula.

Table – 3

STOMATAL INDEX

Observation Number	Lower epidermis
1	25
2	24.71
3	27.08
4	25.48
5	24.52
6	26.28
7	25.24
8	24.28
9	23.26
10	24.56

Range	Minimum	Average	Maximum
Lower epidermis	23.26	25.04	27.08

QUANTITATIVE SCHEDULES FOR LEAVES OF *D. cinerea*

Ash Value

The ash values were determined by using air dried powder of the leaf as per the official method.

Total ash

Two grams of the air dried leaf powder was accurately weighed in a platinum crucible separately. The powder was scattered into a fine even layer on the bottom of the crucible and incinerated by gradually increasing the temperature not exceeding 450°c, until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried powder was calculated.

Water soluble Ash

The ash obtained from the total ash procedure was boiled with 25 ml of water for 5 minutes and the insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited for 15 minute at a temperature not exceeding 450°c. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried powder.

Acid insoluble ash

The ash obtained from the total ash was boiled for five minutes with 25 ml of dilute hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, dried and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

Table - 4

ASH VALUES FOR THE LEAVES OF *D. cinerea*

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	8.63	0.69	-
2	9.24	1.04	-
3	8.41	0.67	-
4	9.55	1.08	-
5	8.84	0.75	-
6	10.02	-	7.69
7	9.44	-	6.28

8	8.78	-	6.02
9	9.62	-	6.59
10	9.86	-	5.92

Range			
Minimum	8.41	0.67	5.92
Average	9.24	0.85	6.5
Maximum	10.02	1.08	6.5

Determination of Loss on Drying

For the determination of loss on drying, the method described by wallis was followed.

One gram of the powdered leaf was accurately weighed in a tarred Petri dish, previously dried under the conditions specified in IP '96. The powder was distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 – 105^0 c for 1 hour. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

Table - 5

PERCENTAGE LOSS ON DRYING FOR THE LEAVES OF *D. cinerea*

Observation Number	**Loss on Drying (%) W/W**
	Leaf
1	7.78
2	7.45
3	8.48
4	7.68
5	8.06

Material	Minimum	Average	Maximum
Leaf	7.45	7.89	8.06

EXTRACTIVE VALUES

Petroleum Ether Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of petroleum ether in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ºC and weighed. The percentage of the petroleum ether soluble extractive value was calculated with reference to the air dried powder.

Benzene Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of benzene in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of benzene. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ºC and weighed. The percentage of the benzene soluble extractive value was calculated with reference to the air dried powder.

Ethyl Acetate Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of ethyl acetate in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethyl acetate. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105ºC and weighed. The percentage of the ethyl acetate soluble extractive value was calculated with reference to the air dried powder.

Chloroform Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of

chloroform in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the chloroform soluble extractive value was calculated with reference to the air dried powder.

Ethanol Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of ethanol in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethanol. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the ethanol soluble extractive value was calculated with reference to the air dried powder.

Water Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of chloroform water in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform water. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the chloroform water soluble extractive value was calculated with reference to the air dried powder.

Table - 6

EXTRACTIVE VALUES (INDIVIDUAL SOLVENTS)

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	0.93
Benzene	1.08
Ethyl acetate	0.9

Chloroform	1.09
Ethanol	2.75
Water	4.33

Extractive Values

By using solvents successively with increasing order of polarity

Five grams of the coarsely powdered leaf was extracted continuously in soxhlet apparatus for six hours individually, separately with solvents of increasing order of polarity. After six hours the solvents was removed and evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°c and weighed. The percentage of the individual solvent soluble extractive value was calculated with reference to the air dried powder.

Table – 7

EXTRACTIVE VALUES (SUCCESSIVE SOLVENTS)

Solvents	**Extractive Value (%)**
	Leaf
Petroleum ether	1.52
Benzene	1.86
Ethyl acetate	1.02
Chloroform	1.24
Ethanol	1.50
Water (reflux)	4.01

Chapter - V

PHYTOCHEMICAL STUDIES

SECTION – A

The plant **D. cinerea** was collected at dry forests area of Perambalur District in Tamilnadu during the first week of August and it was Authenticated by the Taxonomist. The leaves portion was washed thoroughly and dried in shadow. The shadow dried leaves were powdered separately and then subjected to the following preliminary phytochemical studies.

ORGANOLEPTIC EVALUATION

Nature of the Powder	:	Coarse
Colour	:	Green
Odour	:	Distinct odour
Taste	:	Intensively bitter
Shaken with Water	:	Frothing occurs
Pressed in between two filter paper	:	No oily mark on the paper.

PHYTOCHEMICAL STUDIES FOR THE LEAF POWDER OF *D. cinerea84.*[85.86.87]

Powdered Materials and their individual extracts obtained from different increasing

polarity were subjected to the following chemical test and the results were presented in the table.

Test for Alkaloids

Various procedures to liberate alkaloids

- Powdered drug was mixed thoroughly with 1 ml of 10% ammonia solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was mixed thoroughly with 1 ml of 10% sodium carbonate solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was ground in a mortar for about 1 minute with 2 ml of 10% ammonia solution and then thoroughly mixed with 7 gram basic aluminum oxide. The mixture was then loosely packed in to a glass column and 10 ml chloroform was added, eluted, dried and methanol was added.
- Powdered drug was shaken for 15 minute with 15 ml of 0.1 N sulphuric acid and then filtered. The filter was washed with 0.1 N sulphuric acid to a volume of 20 ml filtrate; 1 ml concentrated ammonia was then added. The mixture was then shaken with two portions of 10 ml diethyl ether. The ether was dried over anhydrous sodium sulphate, filtered and evaporated to dryness and the resulting residue was dissolved in methanol.
- Powdered drug was mixed with one gram of calcium hydroxide and 5 ml of water, made into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. 20 ml of 90% alcohol was added, mixed well and then refluxed for half an hour on a water bath. It was then filtered and the

alcohol was evaporated. To that dilute sulphuric acid was added.

The above made extracts were tested with various alkaloid reagents and the results were as follows.

1. Mayer's reagent	-	No cream color precipitate
2. Dragendorff's reagent	-	No reddish brown precipitate
3. Hager's reagent	-	No yellow precipitate
4. Wagner's reagent	-	No reddish brown precipitate

Test for purine group (Murexide test)

The residue obtained after the evaporation of chloroform was treated with 1 ml of hydrochloric acid in a porcelain dish and 0.1g of potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapours of dilute ammonia solution.

No purple colour was obtained indicating the **absence** of purine group of alkaloids.

Test for Carbohydrates

- ***Molisch's test***

The aqueous extract of the powdered material was treated with alcoholic solution of α - naphthol in the presence of sulphuric acid**.**

Purple colour was obtained indicating the **presence** of carbohydrates.

- ***Fehling's test***

The aqueous extract of the powdered material was treated with Fehlings I and II solution and heated on boiling water bath.

Reddish brown precipitate was obtained indicating the **presence** of free reducing sugars.

- ***Benedict's test***

The aqueous extract of the powdered drug was treated with Benedict's reagent and

heated over a water bath.

Reddish brown precipitate was obtained indicating the **presence** of reducing sugars.

Test for Glycosides

General test

❖ ***Test A***

200 mg of the powdered drug was extracted with 5 ml of dilute sulphuric acid by warming on a water bath, filtered and neutralised with 5% sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it becomes alkaline and heated on a water bath for 2 mts.

❖ ***Test B***

200 mg of the powdered drug was extracted with 5 ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it becomes alkaline and heated on a water bath for 2 mts.

The quantity of red precipitate formed in test A is greater than in test B indicating the **presence** of glycosides.

❋ ***Anthraquinones***

❖ ***Borntrager's test***

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The inorganic layer was separated and ammonia solution was added slowly.

No color reaction was observed in ammoniacal layer indicating the **absence** of anthracene derived glycosides.

❖ ***Modified Borntrager's test***

About 0.1 gram of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and

cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well.

No color was observed in ammoniacal layer indicating the **absence** of anthracene derived glycosides.

Test for cyanogenetic glylosides

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place.

No change in the colour of the sodium picrate paper was observed indicating the **absence** of cyanogenetic glycosides

❋ ***Test for cardiac glycosides***

❖ ***Keller Killiani test***

About 1 gram of the powdered leaf was boiled with 10 ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10 ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid containing a trace of ferric chloride. To this 3 ml of concentrated sulphuric acid was added to the sides of the test tube carefully.

No reddish brown layer acquiring bluish green color after standing was observed indicating the **absence** of deoxy sugars of cardiac glycosides.

❖ ***Raymond Test***

To the alcoholic extract of the leaf, hot methanolic alkali was added.

No Violet color was produced indicating the **absence** of cardiac glycosides.

❖ ***Legal's Test***

To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro pruside solution were added.

No blood red color was formed indicating the **absence** of cardiac glycosides.

Coumarin glycosides

A small amount of powdered leaf was placed in test tube and covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes. Then the paper was removed and exposed to UV light.

No green fluorescence was observed indicating the **absence** of coumarin glycosides.

Test for Phytosterols

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

❖ ***Salkowski Test***

Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside.

The chloroform layer of the solution turned red in color indicating the **presence** of sterols.

❖ ***Libermann – Burchard's Test***

To the chloroform solution few drops of acetic anhydride was added and mixed well 1 ml of concentrated sulphuric acid was added through the sides of the test tube and set aside for a while.

A brown ring was formed at the junction of the two layers and the upper layer turned

green indicating the **presence** of sterols.

Test for Saponins

About 0.5 gram of the powdered leaf was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously**.**

Frothing occurred indicating the **presence** of saponins.

Test for Tannins

To the aqueous extract of the powdered leaf, few drops of ferric chloride solution were added.

Bluish black color was produced, indicating the **presence** of tannins.

- ***Gold beater's skin test***

2% hydrochloric acid was added to a small piece of gold beater skin and rinsed with distilled water and placed in the solution to be tested for five minutes. Then washed with distilled water and transferred to a 1% ferrous sulphate solution.

Formation of brown color indicates the **presence** of tannins.

Test for Proteins and Free Aminoacids

- ***Millon's test***

The aciduous alcoholic extract of the powdered leaf was heated with Millon's reagent.

The colour was changed to red on heating indicating the **presence** of proteins.

- ***Biuret test***

To the alcoholic extract of the powdered leaf 1 ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added.

Violet color was obtained indicating the **presence** of proteins.

- ***Ninhydrin Test***

To the extract of the powdered drug, ninhydrin solution was added, and boiled.

Formation of violet color indicating the **presence** of Aminoacids

Test for Mucilage

To the aqueous extract of the powdered leaf, ruthenium red solution was added.

No Reddish pink color was produced indicating the **absence** of Mucilages.

Test for Flavonoids

- ***Shinoda Test***

A little amount of the powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 minutes.

Purple color was not obtained indicating the **absence** of flavonoids.

- ***Alkaline reagent test***

To the alcoholic extract of the powdered leaf, few drop of sodium hydroxide solution was added.

Yellow color was not formed, indicating the **absence** of flavonoids

- ***Zinc Hydrochloride Test***

To the alcoholic extract, mixture of zinc dust and concentrated hydrochloric acid was added.

No formation of red color indicating the **absence** of flavonoid

Test for Terpenoids

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform. To the chloroform solution tin and thionyl chloride were added.

Pink color was obtained indicating the **presence** of Terpenoids.

Test for Volatile Oil

About 100 gram of fresh leaves, were taken in a volatile oil estimation apparatus (Cocking Middleton apparatus) and subjected to hydro distillation for four hours.

No Volatile oil was obtained indicating the **absence** of volatile oil.

Test for Fixed Oil

A small amount of the powdered leaf was pressed in between in the filter paper and the paper was heated in an oven at 105^{o}C for 10 minutes.

No translucent greasy spot occurred indicating the **absence** of fixed oil.

Table - 8

RESPONSE TO THE PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE CRUDE LEAF POWDER OF *D. cinerea*

S.NO	TEST	OBSERVATION
I.	**ALKALOIDS**	
	Mayer's reagent	-
	Dragendorff's reagent	-
	Hager's reagent	-

	Wagner's reagent	-
II	**CARBOHYDRATES**	
	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
III	**GLYCOSIDES**	
	General Test	+
	Anthraquinone	-
	Cardiac	-
	Cyanogenetic	-
	Coumarin	-
IV	**PHYTOSTEROLS**	
	Salkowski test	+
	Liberman Burchard's test	+
V	**SAPONINS**	+
VI	**TANNINS**	+
VII	**PROTEINS AND FREE AMINO ACIDS**	
	Millon's test	+
	Biurett test	+
VIII	**GUMS AND MUCILAGE**	-
IX	**FLAVONOIDS**	
	Shinoda test	-
X	**TERPENOIDS**	+
XI	**VOLATILE OIL**	-
XII	**FIXED OIL**	-

The above described tests were also performed on the different extracts of leaf powder of ***D. Cinerea*** and the results were as follows,

Table – 9

RESPONSE TO THE PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE DIFFERENT EXTRACTS OF LEAF OF *D. Cinerea*

Tests	Petroleum ether extract	Benzene extract	Ethyl acetate extract	Chloroform extract	Ethanolic extract	Aqueous extract
ALKALOIDS						
Mayers Reagent	-	-	-	-	-	-
Dragendorffs reagent	-	-	-	-	-	-
Hagers reagent	-	-	-	-	-	-
Wagners reagent	-	-	-	-	-	-

CARBOHYDRATES						
Molishch's Test	-	-	-	-	+	+
Fehlings Test	-	-	-	-	+	+
Benedicts Test	-	-	-	-	+	+
GLYCOSIDES						
General Test	-	+	-	-	+	+
Anthraquinone	-	-	-	-	-	-
Cardiac	-	-	-	-	-	-
Cyanogenetic	-	-	-	-	-	-
Coumarin	-	-	-	-	-	-
PHYTOSTEROLS						-
Salkowski Test	+	-	+	+	-	-
Libermann Burchard Test	+	-	+	+	-	-
SAPONINS	-	-	-	-	+	+
TANNINS	-	-	-	-	+	+
PROTEINS & FREE AMINO ACID						
Millons test	-	-	-	-	+	+
Biuret test	-	-	-	-	+	+
Ninhydrin test	-	-	-	-	+	+
GUMS & MUCILAGE	-	-	-	-	-	-
FLAVONOIDS						
Shinoda test	-	-	-	-	-	-
Alkaline Reagent test	-	-	-	-	-	-
Zinc hydrochloric acid test	-	-	-	-	-	-
TERPENOIDS	+	+	+	+	+	-
FIXED OIL	-	-	-	-	-	-

"+" Indicate Positive reaction "-" Indicate Negative reaction

Determination of Foaming Index

One gram of the coarsely powdered leaf was weighed and transferred to 500 ml conical flask containing 100 ml of boiling water. The flask was maintained at moderate boiling, at 80-90^{o}C for about 30 minutes. Then it was cooled and filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100 ml (V1)

Ten stoppered test tubes were cleaned (height 16 cm, diameter 1.6 cm) and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3, ml up to 10 ml and

adjusted the volume of the liquid in each tube with water to 10ml. Then the tubes were stoppered and shaken lengthwise for 15 seconds, uniformly and allowed to stand for 15 minutes and measured the length of the foam in every tube.

The length of the foam was less than 1 cm in every tube and hence the Foaming index in **less than 100**.

SECTION – B

THIN LAYER CHROMATOGRAPHY OF ETHANOLIC EXTRACT OF D. cinerea BY USING VARIOUS SOLVENT SYSTEM.[88,89,90]

Among the various methods of separating and isolating plant constituents the "chromatographic Procedure" originated by Tswett is one of the most useful techniques of general application. All finely divided solids have the power to adsorb other substances are capable of being absorbed some much more readily than others. Thin phenomenon of selective adsorption is the fundamental principle of chromatography.

Principle

When a mixture of compound is spotted on a TLC plate the compound which readily soluble and not strongly absorbed moves up readily along with this solvent. Those which are not so soluble, and are more strongly absorbed moves up less readily to the separation of the compound.

The advancement of the TLC techniques has provided the organic chemists and biochemists a tool which combines in itself sensitivity and rapidity compound to the conventional paper chromatographic technique.

Application of substance mixture for separation

The substance mixture was taken in a capillary tube and it was spotted on TLC plated

1cm above its bottom end the start points were equally sized as for as possible.

Development of chromatogram

The plates were developed in a chromatographic tank by using a range of solvents from non-polar to polar as a mobile phase. The plates were allowed to develop ¾ of the length and then removed. The solvent front was immediately marked and the plates were allowed to dry. Then the plates were examined visually or under UV (or) sprayed with different reagents.

The spots were identified and Rf values were determined.

Stationary Phase : TLC Aluminium sheet precoated with slicagel 60 F254. (merck)

Mobile phase used : Hexane : Ethyl acetate 5 : 5

Hexane : Ethyl acetate 2 : 8

Ethyl acetate : Ethanol 8 : 2

Detecting Agent : UV CAMAG 254 nm

The Rf value was calculated by the following formula.

$$Rf = \frac{\text{Distance travelled by solute front}}{\text{Distance traveled by solvent front}}$$

TLC of ethanolic extract of **D. cinerea** by using various solvent system are tabulated here.

Table – 10

TLC OF ETHANOLIC EXTRACT OF D. CINEREA LEAF

S. No	Mobile Phase used	Observation	Rf value
1.	Hexane :Ethyl acetate 5 : 5	3 spots obtained. 2 yellow and one green.	0.6, 0.73, 0.77
2.	Hexane :Ethyl acetate 8 : 2	4 spots obtained. 3 yellow and one green	0.20, 0.32, 0.73, 0.8
3.	Ethyl acetate : Ethanol 8 : 2	4 spots obtained. 2 yellow and one orange and one green.	0.2, 0.46, 0.58, 0.8

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY ANALYSIS OF *D.cineea* LEAF EXTRACT DEVELOPMENT OF HPTLC FINGER PRINT[91]

The ethanolic extract of ***D. cinerea*** leaves were applied in a concentration of 10µl using CAMAG Linomat IV sample applicator on Aluminium sheets precoated with silica gel 60 F_{254} HPTLC plates of 0.2mm thick, 5x20cm, used as a stationary phase. The plates were developed in the mobile phase, hexane : Ethyl acetate (7:3) for the ethanolic extract to a distance of 120 mm in CAMAG – Twin trough glass chamber. Then the track was scanned using CAMAG densitometer scanner II equipped with CAMAG software © 1998 CATS 3.20", at a wavelength of 254 nm using deuterium lamp and the finger print profiles were recorded and presented in Table.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)[91]

Instrument	:	CAMAG HPTLC system equipped with.
Applicator	:	CAMAG linomat IV sample applicator
Scanner	:	CAMAG densitometer scanner II: 951016
Software	:	CAMAG – SOFTWARE (C) 1988 CATS 3.20
Developing chamber	:	CAMAG – Twin trough glass chamber
HPTLC Plate	:	Silicagel 60F 254, Merck, pre coated HPTLC plate of 5 x 20cm, 0.2 mm thick.

Solvent systems : 1) hexane : ethylacetate

7 : 3

Wavelenth : 254 nm

Sample : Ethanolic extract of ***D.cinerea leaves***

PROCEDURE

The spots of samples (10µl) were applied about 6mm from the edge of the TLC plates. The plates were developed upto 120 mm in the mobile phase.

HPTLC CHROMATOGRAM

Ethanolic extract - 9 peaks

TABLE - 11

HPTLC Profile of Ethanolic extract of *D.cinerea leaves*

Peak Numbers	R_f	Peak Height	% of total area covered by individual peak
1.	0.05	15.2	40.08
2.	0.17	16.3	13.03
3.	0.32	3.4	2.70
4.	0.39	2.4	1.90
5.	0.48	6.7	5.32
6.	0.62	9.1	7.30
7.	0.71	18.7	14.93
8.	0.83	8.3	6.6
9.	0.93	10.1	8.09

SECTION – C

ISOLATION AND IDENTIFICATION OF ACTIVE CONSTITUENTS IN ETHANOLIC EXTRACT OF D. cinerea BY COLUMN CHROMATOGRAPHY[92,93,94,9,5,96]

Principle

The column consists of narrow-bore tubing packed with a finely divided inert solid that holds the stationary phase on its surface. The mobile phase occupies the open spaces between the particles of the packing. The components distribute themselves between the mobile phase and the stationary phase; elution that occurs by forcing the sample components through the column by continuously adding fresh mobile phase. The average rate at which a solute migrates depends on the fraction of time it spends in that phase. Ideally the resulting differences in rates cause the components in a mixture to separate into bands or cones, along the length of the column.

Methodology

The recent interest in the plant kingdom as a potential source of new drugs envisage alternate strategies for the fractionation of plant extracts rather than on a particular class of compound. Since not all the chemical compounds elaborated by plants are of equal interest to the pharmacognosist. The so called active principles are frequently alkaloids or glycosides and these, therefore, deserve special attention, other groups such as carbohydrates fats and proteins are of dietetic importance and many such as starches and gums are used in pharmacy but lack any marked pharmacological actions.

Other substances such as calcium oxalate, silica, lignin and colouring matters may be assistance in the identification of drugs and the detection of adulteration. The present approach is based on bioactivity guided fractionation and isolation of active compounds from **D.cinerea**

EXTRACTION OF PLANT MATERIALS:

CONTINUOUS HOT EXTRACTION

The healthy leaves of **D.cinerea** was collected during **August 2007** at Perambalur, Tamilnadu near Trichy, washed and dried in the shade. The drug material was powdered sieved, (powder mesh size 60). The powder was defatted with pet ether by continuous hot percolation using soxhlet apparatus then extracted with ethanol (99%) for six hours.

The solvent was removed under reduced pressure. The residue (2.75%) was semisolid **dark green** in colour, **viscous** in consistency.

Material and Method

Weight of extract : 30 gm

Weight of silica gel (Merck) : 150 gm

Solvents used : The GR Grade Solvents were used Sd fine chemicals

Borosil column length and diameter : 48 x 16 cm

BUCHI ROTO VAPOR : V250

The column was packed by wet packing and started the separation by eluting with solvent gradually with increasing order of polarity using hexane and ethyl acetate, iso propyl alcohol (Table-12). All the fraction were collected separately pooled and solvent removed under reduced pressure using rotary evaporator. The fractionation of the compound are tabulated here.

Table – 12

FRACTION OBTAINED IN COLUMN CHROMOTOGRAPHY OF ETHANOLIC EXTRACT OF LEAF OF D.cinerea

S. No.	Fraction No	Eluent	Observation
1.	1-3	Hexane : Ethyl acetate 80 : 20	F1 – Shows no Spot on TLC F2 – Shows 1 Spot

			F3 – shows 3 spots on TLC
2.	4-7	Hexane : Ethyl acetate 60 : 40	F4 – 3 spots obtained
3.	8-10	Hexane : Ethyl acetate 40 : 60	F8 – shows blue spot with terpenoid reagent F9&10 – 2 spots are obtained (similar)
4.	11-12	Hexane : Ethyl acetate 20 : 80	Single spots was obtained
5.	13-15	100% Ethyl acetate	F13 – solid settled, single spot obtained (compound I)
6.	16-17	Ethyl acetate : Iso propyl alcohol (75 : 25)	F16-single spot obtained (compound II)
7.	18-20	Ethyl acetate : Iso propyl alcohol (50 : 50)	Single spot obtained
8.	21-22	Ethyl acetate : Iso propyl alcohol (25 : 75)	F21- solid settled dipped in methanolic .H_2SO_4 and heated appearance of brown colour shows presence of sugar

Identification of Compound

The compound 1 was obtained in 100% ethyl acetate fraction (F 13). It gave single spot with terpenoid reagent.

Compound I (F13)

State : Solid

Color : Pale Yellow

Solidity : It is soluble in mixture of methanol and chloroform.

Character : It is UV inactive.

Yield : 40 mg

The Compound I was answered sugar and terpenoid test.

Thin layer chromatography of Isolated compound I (Plate No-16)

- Mobile phase : 20% ethanol

80% ethyl acetate

- ❖ Detecting Agent : Terpenoid reagent
- ❖ Observation : Blue colour spot was obtained with terpenoid reagent.
- ❖ Rf value : 0.7

The residue was subjected to IR and NMR, Mass spectral studies.

Compound II

It was obtained in the fraction of 75% ethyl acetate and Isopropyl alcohol (F16). It gave single spot on TLC which was pooled and concentrated to get brown solid which was further purified by recolumn using the length and diameter of the column (46cm x 6cm), eluted with various percentage of the solvents.

Table – 13

RE-COLOUMN CHROMOTOGRAPHY OF FRACTION 16

S. No.	Fraction	Percentage of solvent	Observation
1.	1	100% Hexane	No spot was obtained on TLC
2.	2-4	Hexane : Ethyl acetate 50 : 50	No spot was obtained on TLC
3.	5-7	100% Ethyl acetate	7 – light yellow spot was obtained
4.	8-14	Ethyl acetate : Iso propyl alcohol (95:5)	9,10 – shows single spot on TLC
5.	15-20	Ethyl acetate : Iso propyl alcohol (90:10)	-

Fraction 9 -10 shows single spot on TLC which was concentrated to get pure compound II.

Compound II (F16)

State : Solid

Color : Brown

Character : It is low melting point solid.

Yield : 120mg

Thin layer chromatography of Isolated compound II (Plate No-17)

- Mobile phase : 20% ethyl acetate
  80% ethanol
  1ml (aceticacid)
- Detecting Agent : UV light 254 nm
- Observation : Yellow colour spot was obtained After derivatisation with terpenoid reagent it gave blue spot.
- Rf value : 0.63

Characterization and Structural elucidation of Isolated compound

Isolated Compound was further checked by TLC and characterized by 1H, C^{13} NMR, IR and mass spectroscopic techniques. The chemical structure was elucidated and discussed in table and possible structure was given below.

INTERPRETATION OF NMR[97,98,99,100,101,102]

Instrument used : Burker 500 MHz,

Solvent : DMSO d6

Table -14

Groups	δ ppm	Groups	δ ppm
C-1	36.69	H – 1	-
C-2	56.74	H – 2	1.37 (1 H,m)
C-3	38.78	H – 3	1.58 (2H,m)
C-4	38.78	H – 4	-
C-5	23.08	H – 5	1.78 (2H,m)
C-6	77.79	H – 6	3.15 (1H,m)
C-7	39.84	H – 7	-
C-8	33.82	H – 8	1.52 (2H,m)
C-9	51.06	H – 9	1.81 (1H,m)
C-10	19.09	H – 10	1.79 (2H,m)
C-11	140.94	H – 11	-
C-12	42.33	H – 12	-
C-13	121.69	H – 13	5.18 (1H, m)
C-14	23.08	H – 14	1.90 (2H, m)
C-15	31.89	H – 15	-
C-16	25.34	H – 16	1.50 (2H, m)
C-17	50.08	H - 17	2.50 (2H, m)

Groups	δ ppm	Groups	δ ppm
C-18	25.34	H – 18	1.93 (2H, m)
C-19	35.96	H – 19	1.47 (2 H,m)
C-20	37.31	H – 20	1.51 (2H,m)
C-21	31.80	H – 21	-
C-22	45.62	H –22	1.94 (2H,m)
C-23	29.74	H – 23	0.83 (3H,s)
C-24	29.74	H – 24	0.84 (3H, s)
C-25	29.18	H – 25	0.89 (3H,s)
C-26	19.58	H – 26	0.79 (3H,s)
C-27	25.34	H – 27	0.90 (3H,s)
C-28	19.41	H – 28	0.96 (3H,s)
C-29	21.58	H – 29	0.99 (3H,s)
C-30	21.41	H – 30	1.00 (3H, s)
C-31	61.58	H – 31	3.49 (2H, m)
C-32	77.39	H – 32	3.66 (1H, m)
C-33	101.27	H – 33	4.86 (1H, m)
C-34	73.94	H – 34	3.65 (1H, m)
C – 35	77.25	H – 35	3.64 (1H, m)
C – 36	70.58	H – 36	3.64 (1H, m)

m– Multiplet s– Singlet Methyl groups – exchangeable

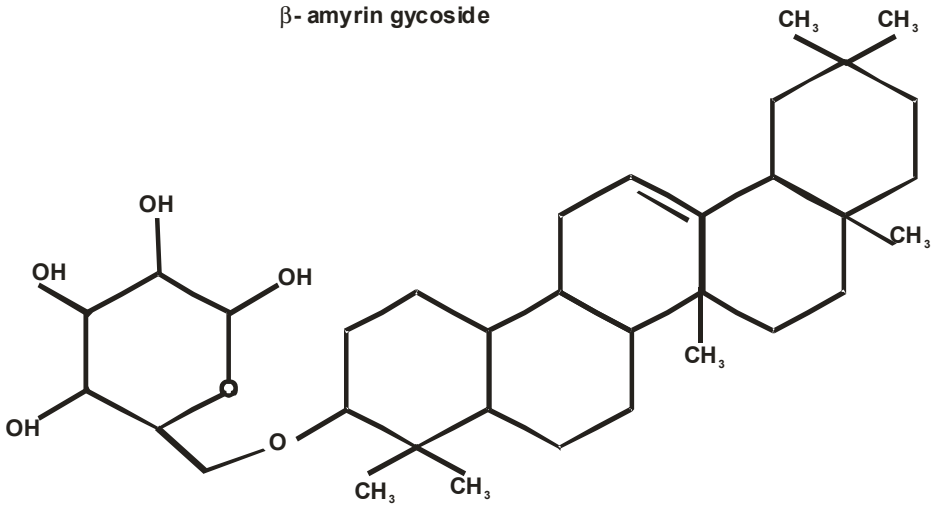
Interpretation of IR

Frequency	Group Assigned
3410	(0H)
2963	(CH2 CH)
2934	(CH3)
1609	(C = C)
1417	(C = C)
1167	(CH)
799	(C = C)
1052	(C – 0)

Mass spectrum (m/e) : 679 (M+1)

The structure of the compound was identified using following techniques like H1, C13, NMR, Mass and IR spectroscopy. The possible structure of the compound may be.

β- amyrin gycoside



Chapter - VI

PHARMACOLOGICAL SCREENING OF ANTI LICE ACTIVITY

SECTION – A

Introduction

The head lice, **Pediculus humanus capitis,** is an obligate ectoparasile of humans that causes pediculosis capitis, a nuisance for millions of people wordwide with high prevalence in children. Although wordwide with high prevalence in children. Although the symptoms are relatively milds has resulted in various social mental and economic problems.[62]

It is estimated that Pediculosis capitis affects 6 -12 million people per year in the united states louse infestation crosses all regional and socioeconomic boundaries, affecting rich and poor alike. Transmission of head lice occurs through close physical contact as well as through fomites such as hats.[59]

About head lice

Lice are wingless, elongated, and dorsoventrally flattened. They are insects with 3 well defined body segments and 3 pairs of clawed legs.

Head lice spend most of their life in the hair, not on the scalp, and come down to the scalp to feed. As feeding usually takes about 15 min or less and they feed about three times a day, head lice really spend only a small amount of their life on the scalp.

Head lice spread easily and infestations often occur at all social and economic levels especially among school children who are in close daily contact. At least 10 million children are infected each year. It is communicable disease.

They vary in colour from dirty white to reddish brown to rust to grayish black in colour. They need a warm, moist habitat. They spread by crawling we have had some reports of a strain that appears to jump. They live by biting and sucking blood from the scalp and can not normally survive for more than 2 days unless they are on the human head.

Life cycle of lice[13,58]

Pediculus humanus capitis, the head louse, is an insect of the order Anoplura and is an ectoparasite whose only host are humans. The louse feeds on blood several times daily and resides close to the scalp to maintain its body temperature.

The life cycle of the head louse has three stages: egg Nymph and adult.

Egg

The eggs are known as nits and are always oval – or tear shaped colour: opaque, size : 0.8 mm by 0.3mm and are glued at an angle to the side of the hair shaft. One louse can lay 150 nits a month (Normal life time). They hatch in about 10 days depending on the climate. Nits need at least 82^{o}F and 70% humidify to incubate.

Nymph

The egg hatches to release nymph. Colour : dull yellow and remains attached

to the hair shaft. The nymph looks like an adult except in size and possession of sexual organ. Nymph mature after three molts and become adults about 7 days after hatching.

Adult

After hatching, the louse takes 3 weeks to grow into an adult. The adult louse is about the size of a sesame seed has 6 legs. They often have a tiny dot on their back.

Length of life cycle

The egg hatches 8-9 days and the nymphal stages take approximately the same length of time. The life cycle takes place therefore, every 18 days. The length of the adult stage in the male is about 10 days and in the female can vary from 9-22 days.

A maximum of about 6-10 nits/eggs are laid each day by each female and the maximum hatch rate has been found to be 88%.

All lice feed blood every 3-6 hours and can only survive about 20-48 hours without a blood meal.

Resistance[16]

The control of human head lice world wide depends primarily on the continued applications of organochlorine (DDT and lindane), organophosphorus (Malathion), carbamate (Carbaryl), pyrethrin, pyrethroid (permethrin & δ - phenothrin) and avermectin, insecticides. Their repeated use has often resulted in the development of resistance, and increasing levels of resistance to the most commonly used pediculicides have caused multiple and excessive treatments, fostering serious human

health concerns. These problems have highlighted the need for the development of selective p.humanus capitis control alternatives.

Treatment[16]

Natural products have been used in traditional medicine for thousands of years and recently have been of increasing interest. Since the costs are usually lower and they are considered less toxic by the public. Here we present a review on plant compounds used for the treatment of head lice.

An overview is given on published evidence on the use of natural product offer promise for new compounds to treat head lice infestation. However the number clinical studies is very limited, and there an urgent need to increase research assessing the effectiveness and safety of promising compounds.

Over the counter natural products should be supported by invitro data and by adequately designed comparative trials that evaluate cure rates and safety.

Method of extraction

Continuous hot extraction

The various concentration of ethanolic extract of leaves of **D.cinerea** were prepared by using distilled water.

20%,40%,60% were used.

Materials and Methods

- Glass vessels
- Nylon mesh (1mm mesh screens)
- Hair tuft
- Test drug

- ❖ Adult head lice

Head lice

A colony of **P.humanus capitis** was collected by combing the hair of 20-25 infected children at the age group of 10-15. Head lice were reared in the glass vessels covered with nylon mesh containing tufts of hairs.

Feeding of head lice[67]

To feed head lice with blood meals they were kept on the lower leg of the human beings and maintain there for 30 minutes. Microscopy examination of the mid-gut confirmed blood ingestion.

Bio assay

The hair tufts was impregnated with appropriate doses for the screening. Control hair tufts receiving the vehicle were also maintained, marketed sample were used as a standard.

Batches of 20 adult **P.humanus capitis** given a human blood meal before the bioassay were placed on each vessel containing few strands of human hair and it was covered with the nylon mesh.

Treated and control and standard were held at 37°c in darkness. Each concentration was maintained at triplicate and number of mortality were recorded for every 30 minutes. Death was defined as lack of movement of limbs and guts and failure to respond when the legs were stroked with forceps and the results were tabulated. (Table No – 16)

Table No – 16

Anti lice activity of ethanolic extract and ethonolic extract with various carrier oils

No.	Lice released	Text Drug	Concentration g/10ml	Mean ± SEM % mortality 60 minutes	Mean ± SEM % mortality 90 minut
	N = 20	Aqueous paste	2	-	3.3333 ± 1.667
	N = 20		4	13.3333 ± 1.667	23.3333 ± 1.667
	N = 20		6	23.3333 ± 1.667	43.3333 ± 1.667
	N = 20	EEDC	2	3.3333 ± 1.667	16.666 ± 1.667
	N = 20		4	23.3333 ± 1.667	45.000 ± 2.887
	N = 20		6	61.6666 ± 2.887	98.333 ± 1.667
	N = 20	EEDC + Coconut Oil	2	23.3333 ± 1.667	47.61± 1.002
	N = 20		4	41.6666 ± 3.333	65.32 ± 2.112
	N = 20		6	98.3333 ± 1.667	-
	N = 20	EEDC + gingelly oil	2	8.333 ± 1.667	18.333 ± 1.667
	N = 20		4	28.333 ± 1.667	48.333 ± 1.667
	N = 20		6	68.3333 ± 1.667	98.333 ± 1.667
	N = 20	EEDC + Castor oil	2	3.3333 ± 1.667	21.666 ± 1.667
	N = 20		4	23.3333 ± 1.667	53.333 ± 1.667
	N = 20		6	65.24 ± 1.667	98.333 ± 1.667
	N = 20	Standard marketed sample	10ml	98.666 ± 0.5774	-

EEDC : ethanolic extract of **D.cinerea**

The percentage lethality was determined by LC 50 value. It was obtained from the graph plotted, concentration vs percentage lethality. (Fig No-10)

Statistical analysis

The values were expressed in mean ± SEM. The observation were made in triplicate. Datas were analyzed by student t.test and $P < 0.05$.

Since the isolated fraction were less quantity, if was not possible to carry out the experiment in triplicate. So the percentage mortality after 60 min and 90 minutes were tabulated. (Table N0-17)

Table - 17

ANTI LICE ACTIVITY OF ISOLATED FRACTIONS

S.No.	Fractions No (Concentration 5%)	Number of lice released	% mortality	
			60 Minutes	90 Minutes
1.	F-4	20	25	50
2.	F-8	20	40	60
3.	F-9	20	60	70
4.	F-16	20	55	65
5.	Standard marketed sample (10ml)	20	98	-

SECTION – B

ANTIDANDRUFF ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF D. CINEREA

Introduction

Dandruff is a major cosmetic problem that posseses very great public health concern both in developed and developing countries . The problem manifests as profuse white to silvery powdery scales in the scalp region often with moderate to severe itching.

Causative organism[20,21,22]

Pityrosporum ovale (Malassezia furfur), a yeast like lipophilic basidiomycetous fungus ,is considered to be the chief cause of the problem. Besides this, **Candida** species is also suspected in the disease process of dandruff. These organism are widely considered to be the commensal flora of the scalp and skin region.

P. ovale converts the sebum lipid into fatty acids and triglycerides. These fatty acids may presumably accelerate hyper proliferation of keratinocytes.

The skin of the scalp contain numerous hair follicles, sudoriferous and sebaceous glands, and a rich complex innervation and vascular network.

Sebaceous glands associated to the hair follicles secrete sebum, which lubricates and protects the scalp and make hair flexible and shiny.

Dandruff is defined as excessive flaking and is associated with mild pruritis without inflammation. When dandruff appears, the normal shedding of epidermis is

accelerated in a way that newly formed cells at the basal layer need half the time to reach the horny layer than of horny cells are shed as large clumps commonly known as flakes.

Types of dandruff

Dry dandruff or Pityriasis simplex

This is the most frequently occurring type of dandruff. In this case, the scalp appears dry and rough.

Oily dandruff (or) Pityriasis steatoids

In this case, desquamation is associated with seborrhea. The hair looks shiny and oily with thick yellowish flakes. It may associated to erythema, prurities and scalp inflammation.

Main causes

1. Environment conditions: Dandruff is usually more severe in winter than in summer.
2. Hyperproliferation of the epidermis.
3. Hormone imbalance

Materials and methods

Petridish, SDA medium (Sabouraud Dextrose Agar), micro pipette, hot air oven, autoclave, incubator.

Composition of the medium

Mycological peptone - 10 gm

Dextrose	-	40 gm
Agar	-	15 gm

Method : Disc diffusion method

Organism used : Malassezia furfur (MTCC:1374)

Preparation of the medium

2gm of SDA medium and 1gm of Agar was dissolved in 50ml of distilled water heat to boiling to dissolve the medium completely, sterilize by autoclaving at 15lbs pressure (121^{o}C for 15 mts p^{H} is adjusted to (5.6 $\pm$ 2^{o}C).

The medium was poured into the sterile petridishes to get a thickness of 5-6mm. The medium was allow to solidify and petridish was inverted and were dried at 37^{o}c just before inoculation.

Inoculum preparation

The peptone was added to the liquid SDM in the concentration of 5,10,15 and 20g/lr. Pure culture of **M. furfur** grown in liquid medium was inoculated and incubated at 30 $\pm$ 2^{o}C for 7 days.

Collection and maintenance of the culture

Pure culture of **M.furfur** (MTCC: 1374) was obtained from institute of Microbial type of culture collection, Chandigarh, India. The culture was maintained in SDA medium.

Antimycotic assay (Disc – diffusion method)[69]

The broth culture of **M.furfur** was swabbed over the sabouraud dextrose agar by using stierile cotton buds. Sterile 5mm diameter whatman No. 32 filter paper discs were dipped in plant extracts and clotrimazole (Standard drug10 µg/disc) and control DMSO disc were placed equidistantly (3cm apart) round the margin of the plates. (Plate No-18,19)

Three replicates were maintained. The plates were incubated at 30 ± 2ºC and zone inhibition was observed after 3 days. The results were tabulated here.

Table No - 18

ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACT OF D.CINEREA AGAINST M.FURFUR

S.No.	Drug	Concentration	Zone of inhibition
1.	Ethanolic extract	50mg/ml	Nil
		100mg/ml	Nil
		250mg/ml	6mm
		500mg/ml	9mm
2.	Standard (clotrimazole)	10µg/disc	15mm
3.	Control DMSO	-	-

Chapter - VII
RESULTS AND DISCUSSION

The dissertation covers the works on pharmacognostic, phytochemcial preliminary invitro antilice and anti dandruff screening of **D.cinerea,** in an attempt to rationalize its use as single drug of therapeutic importance with dual benefit.

Chapter I: Introduction: We discussed the importance of medicinal plants → Plants in the World → Herbs in the World → herbs in India → herbs in modern medicine → role of W.H.O and herbal medicines → natural products in new drug development → priorities in medicinal plant research on infestation → history about lice→ transmission → lice burden → current treatment of head lice → resistance → some natural products used for treatment of head lice→ dandruff – global view → causative organism → symptom of dandruff → Treatment option → plants having anti dandruff activity → an integrated treatment to cure lice & dandruff → reason for the selection of the plant **D. cinerea**.

Chapter II: Review of Literature : The various literatures available were categorized under ethono medical, pharmacognostical, phytochemical, pharmacological, biological screening of general, aerial part, leaves, stem bark, wood and wood powder, fruits, pods and seeds, seeds, roots. Various formulations in which **D. cinerea** is the one of the ingredients for the treatment of various diseases world wide were reviewed.

Life cycle of **P.humanus** capitis has been fully reviewed → plants screened for antilice activity → methodology → were reviewed.

Dandruff and screening methods for dandruff were also reviewed.

Chapter III: Aim and Objective: Were set to obtain the potential benefit to treat both lice and dandruff with single medicinal plant. Moreover to study **D.cinerea** systematically for proper identification and to find adulterants and substituents, pharmacognostically & phytochemically.

Chapter IV : Section A : Pharmacognostic Studies[35,39,70]

Macroscopical study including taxonomical position → geographical distribution → the habit and habitat → description of leaves, bark, inflorescence, flowers, pods & seeds → seasons of flowering and fruiting → bio-physical limits → means of propagation → seed collection → seed biology and its pretreatment etc were discussed.

Photograph and line drawings were presented as an establishement of authenticity. (Plate No-1-5, Fig-1)

Morphology of the leaf

Alternate	:	bipinnate,
Size	:	1-1.6cm
Leaflets	:	minute, sessile,12-20 pairs, close to each other
Shape	:	linear (or) strap shaped oblique, subacute, green
Margin	:	entire
Apex	:	acuminate
Odour	:	aggreable distinct odour

Taste : bitter

Texture : soft

Showed sensitive movement after plugging. (Plate No-3)

Bark

Gray (or) light brown furrowed with black spot.

Inflorescence

Short peduncled, dense flowered containing bisexual (yellow formed in a distal half, neuter pink formed in basal half.

Calyx

Membranous, **Corolla:** 2-2.5 cm long **Petals :** 5 valvate, **Ovary :** filiform style truncate stigma, many ovuled.

Pod: Glabrous flat, 5-7.5cm, dark brown twisted. **Seed :** obovate, compressed, deep brown, glassy appearance.

Section B

Deals with the microscopical studies of leaflet to ascertain the arrangement of tissues (Plate No: 6-13). The T.S. of leaflet through midrib and the powder analysis showed the following features.

Shape: elliptical with narrow margin (plate n.6) 150μm thick 40μm of the margin.

Epidermis

Tanniniferous, semicircular with raised outer periclincal walls.

Mesophyll: Not well differentiated into palisade and spongy parenchyma. 8-10 layers of short, rectangular tanniniferous, compact and darkly staining cells.

Vascular bundle

Shape : Midrib bundle in circular and marginal strands are smaller.

Xylem: Occurs as **conical cluster,** Phloem : **Thick arc**, both are **encircled by sclerenclymatous bundle sheath.** Stomata **occur on abaxial side**, **paracytic** (rubiaceous) type with smaller and another larger subsidiary cells. (Plate No – 7)

Venation pattern

Vein termination characterized by one (or) more rectangular sclereides, which have thick walls, circular and dot like simple pits. Unicellular conical trichomes were seen along the margin. (Plate No- 8)

Crystals

Cubical, rhomboidal, rectangular shape calcium oxalate prismatic crystals occur in mesophyll tissue and bundle sheath. (Plate No-9)

Rachis

Shapes: shield shape with adaxial, lateral short wings, shallow median concavity and wide adaxial side.

Vascular bundle

Main system has a thick circular, hollow sclerenchymatous cylinder which encloses 5 prominent bundles in which 3 in abaxial 2 are in adaxial side.

It was clear from the above observation that the stomata were rubiacious which was as nearly all recorded instances of Mimosacae family.

2) In these family, number of genera contain relatively little chlorophyll, and the cells being filled with brown tanniniferous contents. The same was noticed in **D.cinerea** also.

Secretary element are absent. Cells with variously coloured contents occur in the leaf of the number of other genera of this family were also absent.

Inner walls of epidermal cells are not frequently mucilagenous which occur in some species of **Dichrostachys.** Hypoderm were not recorded. Stomata are not uniformely distributed on both sides as in some other species of **Dichrostachys**. Mesophyll contains tanniferous materials as in some other genera. There is no water storage cells as in **Sophora tomentosa** (L.).

A specific rectangular screleids in vein termination was characteristic which is comparable to spool shaped stone cells recorded in upper and lower epidermis of **Prosopis** species.

Crystals are solitary, rhomboidal which are predominant in this family. We have also observed the leaves exhibiting **sensitive movement** after plugging as certain

members of the this family (Plate No-3). So these features supports not only for the identification but also to solve the taxonomical problems.

The anatomy of the leaves of members of the family which exhibit 'sensitive movements' has been studied by Stekbeek & Funlea Steekbeck concluded that the movements are controlled by crystals in the endodermis, each of which in surrounded by a protoplasmic sac and provided with intercellular protoplasmic connections.[108]

Stem :

Shape : circular

Epidermis : elliptical, thickwalled, darkly staining.

Cortex : about 8 layers origin of first phellogen observed

Vascular : cylinder

Shape : wary outline, a characteristic

Gelatinous fibres with outer lignified end inner gelatinous wall were observed. (Plate No. 13).

Pith: Parenchyma cells have dark solid body

Section C deals with the quantitative microscopy interms of microscopic and physical parameters. The vein islet and vein termination number of **D.cinerea** were given (Table – 1).

Parameters	Minimum	Average	Maximum
Vein islet number	10	12.5	15
Vein termination number	6	7.4	9

The stomatal number and stomatal index were studied.(Table – 2,3)

Parameters	Minimum	Average	Maximum
Stomatal number			
Lower epidermis	75	79.9	84
Stomatal index			
Lower epidermis	23.26	25.04	27.08

The physical parameter are as follows from (Table -4-7)

Parameters	Minimum	Average	Maximum
Total ash	8.41	9.24	10.02
Acid insoluble ash	0.67	0.85	1.08
Water soluble ash	5.92	6.5	6.5
Los on drying	7.45	7.89	8.06

Extractive values (individual solvent extraction)

Solvents	Extractive value (%)
Petroleum ether	0.93
Benzene	1.08
Ethyl acetate	0.9
Chloroform	1.09
Ethanol	2.75
Water	4.33

Extractive values (successive solvents)

EXTRACTIVE VALUES (SUCCESSIVE SOLVENTS)

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	1.52
Benzene	1.86
Ethyl acetate	1.02

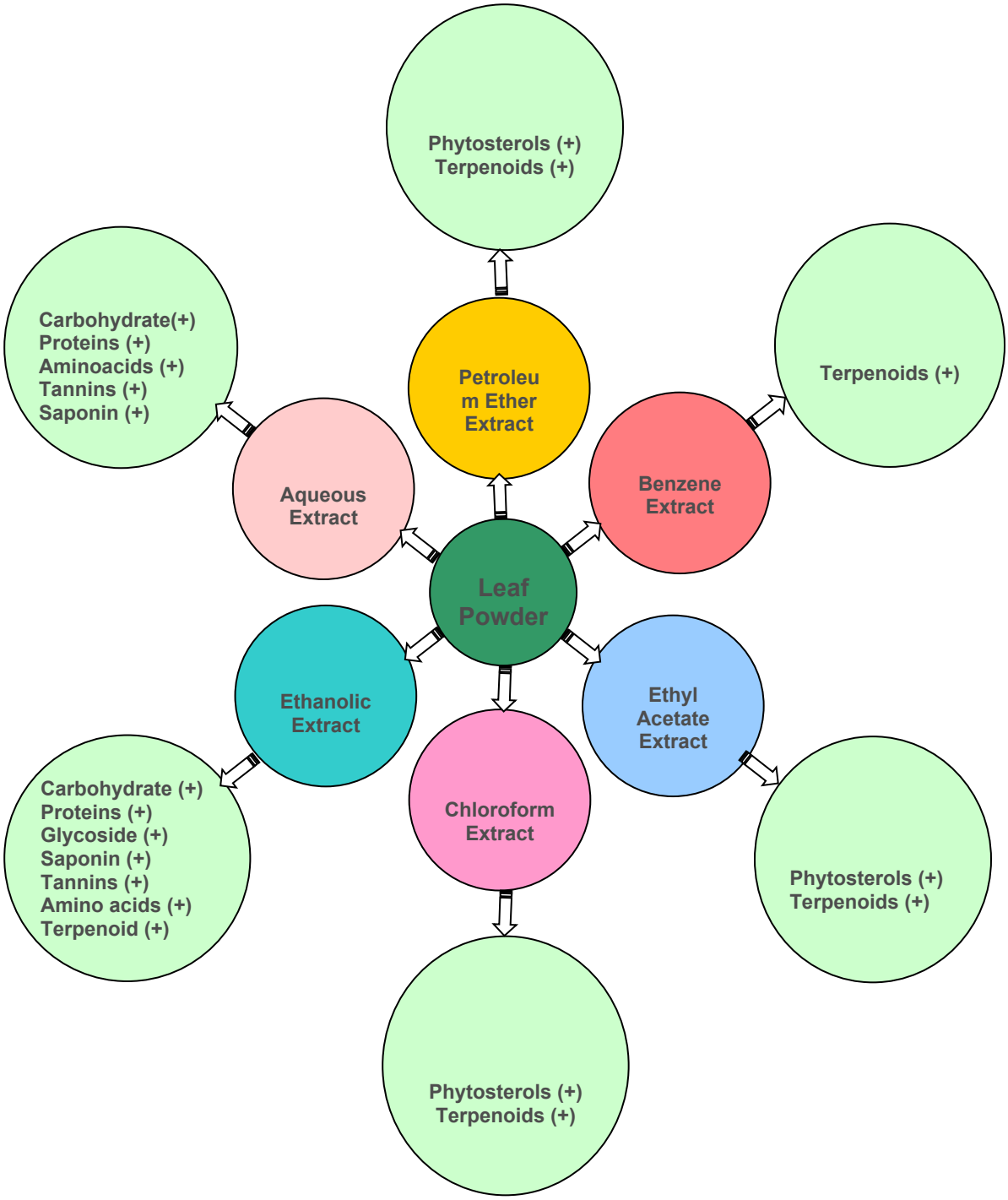
Chloroform	1.24
Ethanol	1.50
Water (reflux)	4.01

Chapter V: Section A: deals with the preliminary phytochemical screening of the powdered leaves and the results were tabulated in (Table No-8).

Constituents present	Constituents absent
Carbohydrates, phytosterols, saponin, glycosides, tannins, proteins & amino acids, terpenoids	Alkaloids, fixed oil gums & mucilage, flavanoids

The foaming index of the powdered mature leaves was <100.

The preliminary screening of various extract obtained by successive extraction of leaf using various solvents of increasing order of polarity was carried out and the results were tabulated in (Table No-9).



Schematic representation of various constituents present in the leaves of **D.cinerea**.

Section B: deals with TLC & HPTLC of the ethanolic extract of leaves of **D.cinerea**.

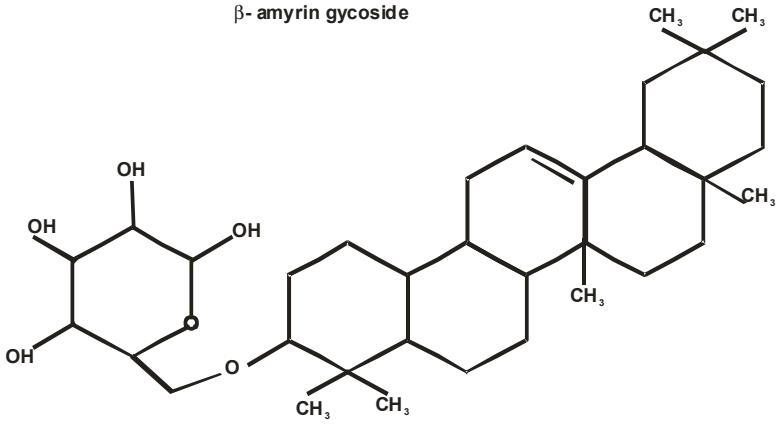
TLC was developed using range of solvents on silicagel $60F_{254}$ as adsorbent. Among the various solvent system the ethylacetate80%/ ethanol20% 4 spots (1 orange, two yellow, one green) observed. The Rf values were 0.2,0.46,0.58,0.8 respectively. (Table No-10, Plate No- 14).

HPTLC profile of ethanolic extract of **D.cinerea** leaves using Hexane – ethylacetate (7:3) showed 9 peaks. (Table No-11, Fig No.-5)

Section C: deals with the isolation and identification of active compound in ethanolic extract of leaves of **D.cinerea** was performed by column chromatography 22 fraction were obtained each of 50ml and the results were tabulated. (Table No-12,13)

The fraction 13 yield solid when crystallized using ethyl acetate. It gave blue color with terpenoid reagent in TLC. The solid subjected to IR, NMR and mass spectral studies and the results were tabulated in (Table no-14,15, Fig No- 6,7,8,9). the possible structure of the compound may be

β- amyrin gycoside



In **Chapter VI**: **Section A:** Though all of the pediculicidal agents acts efficiently against **P.humanus capities,** some of them are neuro toxic. Moreover the continued

use of these products induce resistance. Non toxic alternative options are hence needed for head lice treatments which prompted us this study invitro anti lice activities of and agueous paste, ethanolic extract and along with the fixed oil as carrier and isolated fractions of leaves of **D.cinerea** were studied. (Table No-16,17)

It was observed that after 90 minutes in the percentage mortality observed for the concentration 2,4,6 g/10ml were 3.33 ± 1.667 23.33 ± 43.33±1.66, 16.66±1.66, 45±2.88,98.33±1.66 for aqueous paste, EEDC respectively. It was 47.61 ±1.66, 65.32±2.11 for 2,4 gm/10ml and 18.33±1.66, 48.33 ± 1.66, 98.33 ± 1.66 and 21.66 ±1.66, 53.33 ± 1.66, 98.33 ± 1.66 in the concentration of 2,4,6gm/10ml for EEDC + Coconut oil, EEDC+ Gingelly oil, EEDC + Caster oil respectively.

For EEDC + Coconut oil mortality was significant in an 60 minutes which was 98.33±1.66. All the readings are mean ± SEM for triplicate values.

From the above it is clear that the decrease in percentage of mortality is in the following order after 60 minutes.

EEDC + coconut oil > EEDC + gingelly oil >EEDC+ castor oil >EEDC > aqueous paste, at the concentration of 6 gms/10ml which is comparable to the standard. The ethanolic extract of leaves of **D.cinerea** mixed in coconut oil as carrier possesses significant antilice activity. ($p<0.05$)

Since the isolated fraction were in less quantity, it was not possible to carry out the experiment in triplicate. So the percentage mortality after 60 minutes and 90 minutes were tabulated as. (Table No-17)

From the above table it was observed that the decrease in mortality in the biologically active isolated fractions where as follows at 5% concentration.

F-9 >F-16>F-8>F-4 showed moderate activity and it can be assumed that no single fraction has shown significant mortality. So this study revealed that the combination of phytoconstituents present in the ethanolic extract of leaves of **D.cinerea** posses combined anti lice activity than the isolated fractions.

Section B

Previously it was reported that, the n-hexane and chloroform extracts of aerial part of **D.cinerea** showed antifungal activity against Aspergillus niger and Mucor species at 10mg/ml concentration but the chloroform extract showed activity only at higher concentration. This study and the ethnomedical use prompted us to carry out the Antidandruff screening study.

The antidandruff activity of ehanolic extract of **D.cinerea** against **Malassezia furfur** were studied by disc diffusion method, in SDA medium (Plate No.-18) the zone of inhibition was measured. The results were tabulated in table no.

It was observed that the zone of inhibition was moderate when compared to the standard drug (clotrimazole and Zone of inhibition was slightly concentration dependent & not significant and having moderate inhibition. There was mild increase of inhibition by increasing concentration above 500mg/ml[69].

The diameter of the zone of inhibition is influenced by a variety of factors such as diffusibility of the drug, disc concentration, the nature and composition of the medium, its thickness, presence of inhibitory or stimulatory substances, pH of the

medium and the time of incubation. During incubation, the therapeutic agent diffuses out from the disc in all directions. Agents with lower molecular weights diffuse faster than agents with higher molecular weight might be a powerful inhibitor even though it may diffuse only a small zone of inhibition. Moreover, results obtained in vitro often differ from those obtained in vivo. Metabolic processes in the body of a living organism may inactivate or inhibit on antimicrobial compound[109].

Chapter – VIII

CONCLUSION

This dissertation covers the Pharmacognostic parameters of the leaves of **Dichrostachys cinerea** belonging to the Family **Mimosaceae** such as macroscopical, microscopical including powder analysis and physical standards like ash value extractive values etc have been studied and presented.

The tissue arrangement in the leaf was studied and presented.

The characteristic terminal sclereids were observed in the vein terminations. The presence of sclerenchymatous cells of gelatinous inner wall and outer lignified and dark solid bodies in pith cells were noted in the stem , so that the presence of stem powder in the leaf powder may be identified.

The presence of tanniniferous material was noted in the mesophyll. The sensitive movement of leaves which is present is certain member of this family have been observed and presented.

The preliminary phytochemical studies revealed the presence of carbohydrate, phytosterol, saponin, glycoside, tannin, protein and aminao acids, terpenoids.

HPTLC profile was studied and presented.

Bioactive fraction were isolated and one of the fraction contains β amyrin glycoside which was confirmed by IR, NMR & mass studies.

The anti lice activity against **Pediculus humanus capitis** (head lice) after 90

minutes, of the ethanolic extract of leaves of **D.cinerea** in the concentration of 6gm/10ml was studied. The mortality is concentration dependent. It was further observed that the time for mortality from 90 minutes is reduced to 60 minutes when the extract was mixed with coconut oil as carrier oil.

Application of aqueous paste not possess significant antilice activity. Isolated fractions were tested showed that the activity may be synergistic by the presence of various Phytoconstituents. We were also able to demonstrate invitro moderate anti dandruff activity of the ethanolic extract leaves of **D.cinerea** against M.furfur.

The present study provide scientific basis for the ethnomedical use of this plant as antilice application.

It is concluded that it can be optimistic that the present work proved **D.cinerea** of dual therapeutic advantage to be a potential phytochemical target in the design of a single drug for the treatment of both lice and dandruff without causing any adverse influences on eyes, to alleviate the suffering the affected individuals.

The above study require further investigation for the exact mechanism of action, to develop safe herbal formulation which can result in complementary to those existing pediculocidal agent, which are though acts efficiently they are neurotoxic and induced resistance. So this provide a non toxic alternative options for both head lice and dandruff activities.

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